

CENTRAL HEMODYNAMIC ADJUSTMENTS TO HEMORRHAGE
MEDIATED BY
BETA₂-ADRENERGIC VASCULAR CONTROL MECHANISMS

An experimental study in the cat

By
David Gustafsson

Lund 1984



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Abstract The functional significance of β_2 -adrenergic vascular control mechanisms for central hemodynamic adjustments during graded acute hemorrhagic hypovolemia was studied in anaesthetized cats with intact and selectively blocked β_2 -adrenoceptors (ICI 118,551 used for β_2 -blockade). During a 2 h observation period after bleeding, both groups of animals developed similar arterial hypotension, but other variables were distinctly different. In animals with intact adrenoceptors, there was a gradual partial recovery of plasma volume (PV), stroke volume (SV), and cardiac output (CO) and a gradual restoration to control of an initially raised total peripheral resistance (TPR). In animals with blocked β_2 -adrenoceptors, PV was much less restored, SV and CO remained low, and TPR high. The compensatory PV expansion seemed to be the main cause of the recovery of SV and CO in animals with intact adrenoceptors and it apparently resulted from a marked net trans-capillary fluid absorption in skeletal muscle, a process previously shown to be effectively governed by β_2 -adrenergic microcirculatory adjustments. The gradual restoration of TPR in these animals was mainly accomplished by a β_2 -adrenergic dilator interaction with vasoconstrictor influences on the systemic resistance vessels. This effect tends to improve tissue perfusion in bleeding and, in addition, might facilitate the expulsion of blood from the heart via decreased cardiac afterload, a concept corroborated by studies of central hemodynamic effects of adrenaline, the main endogenous β_2 -adrenoceptor agonist, on normovolemic cats. Studies, with the microsphere technique, of β_2 -adrenergic effects on regional vascular resistances in various organs showed a tendency of a preferential β_2 -adrenergic dilator control in the kidney, pancreas, adipose tissue, and the GI-tract in bleeding, yet without any major β_2 -adrenergic redistributing effect on CO. The survival time after standardized fatal hemorrhage was significantly shorter (38%) in cats with blocked than with intact β_2 -adrenoceptors. It is concluded that β_2 -adrenergic influences on the peripheral and central circulation are highly essential in the organism's defence against bleeding and that they might be crucial for survival after severe hemorrhage.			
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CONTENTS

INTRODUCTION	5
METHODS.....	9
RESULTS AND COMMENTS.....	13
1. Role of β_2 -adrenergic mechanisms in the control of plasma volume restitution in hemorrhage.....	13
2. Influences of β_2 -adrenergic vascular control mechanisms on central hemodynamics in hemorrhage.....	18
3. Central hemodynamic effects of adrenaline with special reference to β_2 -adrenergic influences on heart rate and cardiac afterload.....	28
4. Microsphere analysis of β_2 -adrenergic control of resistance in different vascular areas in hemorrhage.....	35
5. Decrease in survival time in β_2 -adrenoceptor blocked cats exposed to fatal bleeding.....	39
DISCUSSION AND CONCLUSIONS.....	44
SUMMARY.....	47
ACKNOWLEDGEMENTS.....	50
REFERENCES.....	51

This thesis is based on the following publications:

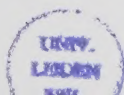
- I. HILLMAN, J., GUSTAFSSON, D. & LUNDVALL, J. 1982. β_2 -adrenergic control of plasma volume in hemorrhage. Acta Physiol Scand 116:175-180.
- II. GUSTAFSSON, D., HILLMAN, J. & LUNDVALL, J. 1982. Influences on central hemodynamics in hemorrhage of β_2 -adrenergic vascular control mechanisms. Acta Physiol Scand 116:181-188.
- III. GUSTAFSSON, D. & LUNDVALL, J. 1984. β_2 -Adrenergic vascular control in hemorrhage and its influence on cardiac performance. Am J Physiol 246:H351-H359.
- IV. GUSTAFSSON, D. & BJÖRKMAN, J.-A. 1984. Central hemodynamic effects of adrenaline with special reference to β_2 -adrenergic influences on heart rate and cardiac afterload in anesthetized cats. Acta Physiol Scand, submitted.
- V. GUSTAFSSON, D., ANDERSSON, L., MÅRTENSSON, L. & LUNDVALL, J. 1984. Microsphere analysis of β_2 -adrenergic control of resistance in different vascular areas after hemorrhage. Acta Physiol Scand 121: 119-126.
- VI. GUSTAFSSON, D., ANDERSSON, L.O. & LUNDVALL, J. 1984. Decrease in survival time in β_2 -adrenoceptor blocked cats exposed to bleeding. Acta Physiol Scand, in press.

In the text, these papers are referred to by their Roman numerals.

INTRODUCTION

It is well established that reflex activation of the sympatho-adrenal system plays a major role in the acute defense of the organism against hemorrhagic hypovolemia. It causes a prompt constriction of the capacitance vessels which tends to adapt the dimension of the circulatory system to the reduced blood volume, and a constriction of the spleen which, in some species, leads to significant recruitment of red cells. Simultaneous adrenergic constriction of the resistance vessels and excitation of the heart contribute importantly to recovery from the initial arterial hypotension. Adrenergic mechanisms are also known to be involved in the most important compensatory reaction to regain a steady state, viz. the replacement of the shed blood volume. In the acute state, such intravascular refill is mainly dependent upon expansion of plasma volume, since the restoration of red cell volume by increased erythropoiesis is a very slow process.

Previous studies have revealed that several mechanisms may contribute to the plasma volume restoration in hemorrhage. Most important, perhaps, for the early replenishment is the transcapillary absorption of extravascular fluid into the circulatory system accomplished by a reflex decrease in capillary hydrostatic pressure mediated by adrenergic resetting of the pre-/postcapillary resistance ratio (Mellander 1960, Öberg 1964). It has been established that reflex sympatho-adrenal activation in the cat, evoked by baroreceptor unloading, chemoreceptor activation, or by hemorrhage, caused such mobilization of extravascular fluid mainly from the large extravascular fluid reservoir of skeletal muscle (Öberg 1964), a finding confirmed in several other species, including man (e.g. Lundgren, Lundwall & Mellander 1964, Mellander & Öberg 1967, Djojosingito, Folkow & Kovách 1968, Schwingamer, Grega & Haddy 1970, Hall, Schwingamer & Lalone 1976). Compensatory fluid shifts from the extravascular to the intravascular compartment can also be evoked in hemorrhage by a transcapillary glucose-osmotic driving force established via arterial plasma hyperosmolality resulting from reflex sympathetic and hormonal release of glucose from the liver (Järhult et al. 1972, Järhult 1975). This mechanism seems to be of special importance in severe bleeding, although it operates gradedly in hemorrhagic hypotension (Järhult et al. 1976). If the fluid content in the gut is sufficient, fluid absorption from the lumen of the small intestine might also occur



as a result of a recently postulated sympatho-adrenal influence on transepithelial net sodium transport (Redfors 1983, Redfors & Sjövall 1984). More gradual and indirect plasma volume restitution is accomplished by renal fluid conservation via various reflex and endocrine mechanisms (Gauer & Henry 1963, 1976, Laragh & Sealey 1973, Pirkle & Gann 1976) and by control of the fluid intake via activation of the thirst mechanism (cf. Fitzsimons 1972).

Most of the research concerning the sympatho-adrenal control of the circulatory system in hemorrhage has been devoted to the evoked excitatory reactions, i.e. the α -adrenergic vasoconstrictor effects in the different vascular circuits and the β_1 -adrenergic effects on the heart. Even if it is well-known that adrenaline can elicit β -adrenergic vasodilator effects in several organs, few attempts have been made to define the physiological role of sympatho-adrenal inhibitory β -adrenergic vascular reactions in bleeding. Yet, it is quite possible that such inhibitory influences significantly can modify the excitatory vascular effects which, in turn, might have important consequences for central cardiovascular hemodynamics in hemorrhagic hypovolemia. This problem was approached in the present studies.

Previous work from this laboratory has demonstrated that the β -adrenergic component of the sympatho-adrenal system contributes importantly to the transcapillary fluid absorption process in cat skeletal muscle during bleeding (Lundvall & Hillman 1978, Hillman 1981). The β -adrenergic control was shown to be mainly confined to the microcirculation and it governed the fluid absorption process by reinforcing the α -adrenergically evoked decrease in capillary hydrostatic pressure and, further, caused a simultaneous increase in functional capillary surface area available for fluid exchange. The capillary pressure fall was accomplished by a resetting of the pre-/postcapillary resistance ratio, in turn caused by a β -adrenergic dilator interaction with concomitant α -adrenergic constrictor influences on the resistance vessels. This dilator effect, in relative terms, was more pronounced in the postcapillary than the precapillary resistance section. The increase in functional capillary surface area was caused by a β -adrenergic relaxation of the precapillary 'sphincters', which significantly promoted the fluid absorption process in hemorrhage (Lundvall & Hillman 1978, Hillman 1981, Lundvall, Hillman & Gustafsson 1982). The β -adrenergic control of fluid absorption was

strictly graded in relation to the extent of bleeding for 10, 20, and 40% reductions of the animal's total blood volume (Hillman 1981).

These β -adrenergic effects were mediated not only via catecholamine release from the adrenals but also to some extent via the sympathetic nerve fibers (Lundvall & Järhult 1976, Hillman & Lundvall 1981 a), confirming and extending the original observation by Viveros, Garlick & Renkin (1968) of a neural β -adrenergic vasodilator response. The β -adrenergic postjunctional microvascular dilator responses were analysed with regard to β_1 - or β_2 -adrenoceptor specificity with the aid of 'selective' receptor agonists and antagonists (Hillman & Lundvall 1981 b). The conclusion was reached that the neural as well as the humoral β -adrenergic control of the microcirculation in muscle was exerted via activation of β_2 -adrenoceptors. This field of research was recently reviewed (Hillman 1983).

In addition to the described control of the capillary exchange function in skeletal muscle there was also a β -adrenergic regulation of total regional resistance and, hence, of blood flow in this tissue during bleeding, exerted via dilator interaction with the vasoconstrictor influences (Hillman & Lundvall 1980). A similar β -adrenergic influence on the resistance function was observed also in some other hemodynamically important vascular areas, viz. the intestine, skin, and to some extent in the kidneys (Lundvall & Gustafsson 1981).

The described β_2 -adrenergic control of microvascular reactions and of the transcapillary fluid absorption process might be expected to have important consequences for overall cardiovascular homeostasis in hemorrhage. In the present studies attempts were made to define more precisely the functional significance of the β_2 -adrenergic vascular control for the central hemodynamic adjustments to acute hypovolemia. This was done by performing comparative studies of various central hemodynamic variables during standardized graded blood loss in cats with intact and selectively blocked β_2 -adrenoceptors, using the highly selective β_2 -adrenergic blocking agent, ICI 118,551, as a pharmacological tool in the analysis.

Such comparative analyses during hemorrhagic hypovolemia led to an evaluation of the importance of β_2 -adrenergic control mechanisms for

1) the compensatory plasma volume restitution (Results, section 1), 2) cardiac output, stroke volume, heart rate, arterial pressure, and total peripheral resistance (section 2), and 3) regional vascular resistances and cardiac output distribution to the tissues (section 4). The central hemodynamic effects of the main endogenous β_2 -adrenoceptor agonist, adrenaline, were also described in normovolemic cats with special reference to β_2 -adrenergic influences on heart rate and cardiac afterload (section 3). The results of these studies, taken together, indicated significant beneficial cardiovascular effects in bleeding of the β_2 -adrenergic control system, which prompted a study of its influence on survival time in cats exposed to fatal hemorrhage (section 5).

METHODS

Detailed descriptions of the methods employed in the present series of expts were given in the original papers (I-VI). Below follows a brief summary of the principal methodological arrangements.

MATERIAL AND ANAESTHESIA

The expts were performed on cats of both sexes (bwt 2.0-5.4 kg) anaesthetized i.v. with urethane (100 mg x kg bwt⁻¹) and α -chloralose (60 mg x kg bwt⁻¹). This anaesthesia is considered to interfere less with cardiovascular reflexes than most other forms of anaesthesia (Armstrong, Porter & Langston 1961, Greisheimer 1965, Warren & Ledingham 1978). Body temperature was kept at $38 \pm 0.5^\circ \text{C}$. Heparin was given i.v. before intravascular instrumentation (1000 IU x kg bwt⁻¹, except in some expts reported in paper IV).

MAIN EXPERIMENTAL PROTOCOLS

The main objective of the present studies (papers I-III, V) was to investigate how β_2 -adrenoceptor controlled cardiovascular effects influence central hemodynamic variables during hemorrhage. For this purpose the central hemodynamic response pattern to hemorrhage was compared in animals with intact and selectively blocked β_2 -adrenoceptors.

Hemorrhage in these expts was accomplished by rapid (<3 min) exsanguination of a fixed volume of blood. Central hemodynamic observations were performed in the prehemorrhagic control period and for up to about 2 h after the acute bleeding procedure. Studies were made during graded hemorrhage, usually 5, 10, and 20 ml x kg bwt⁻¹, corresponding to about 10, 20, and 40% of the animal's total blood volume (cf. paper I).

β_2 -adrenoceptor blockade was instituted by intravenous administration of the highly selective β_2 -adrenoceptor antagonist ICI 118,551 [erythro-dl-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; Imperial Chemical Industries Ltd., England]. This agent, which is reported to be devoid of partial agonist activity but to have membrane stabilizing properties similar to those of propranolol, has a β_2 -selectivity index of 1.7-2.1 (O'Donnell & Wanstall 1980, Bilski et al. 1983). This implies that the dose required to produce a given degree of blockade is 50-125 times smaller for β_2 - than for β_1 -adrenoceptors. The used dose of 75-80 μg x kg bwt⁻¹ (supplemented every h with 30 μg x kg bwt⁻¹ to maintain roughly constant plasma concentration) caused effective β_2 -adrenoceptor blockade (paper III) and has been shown not to interfere with the β_1 -adrenoceptors of the heart (Bilski et al. 1983, Smith, Halliday & Rouse 1983), which was strongly indicated also in the present study (III).

A special investigation of the central hemodynamic effects of the main endogenous β_2 -adrenoceptor agonist, adrenaline, was described in paper IV with special reference to its β_2 -adrenergic influences on heart rate and cardiac afterload. Observations were here made during graded i.v. adrenaline, and also noradrenaline, infusions on normovolemic animals, using both a β_2 -selective (ICI 118,551) and a nonselective β -adrenoceptor blocking agent (propranolol, 500 μg x kg⁻¹) as pharmacological tools in the analysis.

In paper VI an attempt was made to determine to what extent β_2 -adrenergic effects might influence the survival time of cats exposed to fatal

bleeding. Hemorrhage in these expts was accomplished by abrupt withdrawal of 45% of the animal's total blood volume followed by additional withdrawal of $1 \text{ ml} \times \text{kg}^{-1} \times \text{h}^{-1}$ (for chemical analyses) throughout the time of survival.

EXPERIMENTAL PROCEDURES FOR THE STUDY OF CENTRAL HEMODYNAMICS

Three principal types of preparations were used and these are shortly described below.

'Closed-chest' preparation (papers I-IV, VI)

This preparation was used for observations of changes of plasma volume (PV), cardiac output (CO), stroke volume (SV), heart rate (HR), mean arterial blood pressure (MAP), and total systemic peripheral resistance (TPR) evoked by graded hemorrhage or catecholamine infusions on animals with intact and blocked β -adrenoceptors.

Breathing was spontaneous in most animals (I-IV); in some (VI) spontaneous ventilation was aided by an artificial respiration technique. Intravascular instrumentation on the closed-chest preparations was limited to the insertion of a short shunt circuit into the left common carotid artery (used for hemodynamic recordings, blood sampling, and exsanguination) and of a thin central venous catheter introduced via the right jugular vein (used for tracer injections). Care was taken to avoid damage of the vagal nerve and the baro- and chemoreceptor regions. Observations during occlusion of the common carotid artery shunt demonstrated that the baroreceptor reflex was well preserved after the intravascular instrumentation.

PV was determined with a dilution technique (paper I) using i.v. administered human serum ^{125}I -albumin as the tracer (cf. Groom, Rowlands & Thomas 1965). To avoid blood sampling for PV determination, gamma-radiation was monitored in the arterial blood flowing through a defined section of the carotid shunt circuit, using an external scintillation detector connected to a scalar and linear ratemeter (Tri-carb scintillation spectrometer, model 3022, Packard), operating a Rikadenki B-341 recorder (for details see paper I). Radioactivity was measured continuously for 50 min after each injection of the tracer to ensure that the presented PV values referred to states of complete tracer mixing in the plasma compartment. Arterial hematocrit was measured on blood samples taken from the carotid shunt. The PV measurements in the resting control state were found to agree with other reports in which both plasma and red cell volumes have been measured in the cat (Groom & Rowlands 1958, Farnsworth, Paulino-Gonzalez & Gregersen 1960, Groom et al. 1965, Aarseth & Bø 1972).

CO was measured by the indicator-dilution technique (papers II-IV) using ^{131}I -Hippuran, rapidly injected into the right atrium, as indicator (for details see paper II). The indicator-dilution curve was determined by continuous measurement of the gamma-radiation from the arterial blood passing the carotid shunt circuit. Calculations of CO, i.e. the injected activity divided by the area under the dilution curve after subtraction of the recirculation component, were made with the aid of a computer. Since Hippuran is rapidly eliminated, mainly by renal excretion, from the circulation repetitive CO measurements could be performed without any disturbing rise of background activity. The data for CO refer to the mean value of two (paper IV) or three (papers II, III) measurements performed within 4 min. Resting control CO values showed good agreement with those reported by others using a similar technique on closed-chest cats (cf.

Groom & Rowlands 1958, Parratt 1974).

HR (triggered by the arterial pulse operating a Grass tachograph 7P 4D) and MAP (monitored via a Statham P23 transducer) were recorded on a Grass polygraph. TPR was obtained by dividing MAP (mmHg) by CO ($\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$), and SV ($\text{ml} \times \text{kg}^{-1}$) was obtained by dividing CO by HR.

'Open-chest' preparation (paper IV)

This preparation was used for observations of CO (minus coronary flow), SV, HR, MAP, TPR, left ventricular end diastolic pressure (LVEDP), maximal first derivative of left ventricular pressure (max dP/dt), mean systemic central venous pressure (CVP), and mean left atrial pressure (LAP) during graded i.v. adrenaline infusions on animals with intact and blocked β_2 -adrenoceptors. To facilitate the interpretation of the results, especially of left ventricular inotropy, observations were made not only during spontaneous cardiac rhythm but also at fixed HR ($250 \times \text{min}^{-1}$) accomplished by cardiac pacing.

The cats were ventilated artificially, the chest was opened, and the pericardium incised. A micro-tip pressure transducer (Millar Micro-tip PC350) inserted transmurally into the left ventricle at the apex was used to measure LVEDP and max dP/dt . LAP was monitored from a catheter inserted into the left atrium. Pacing electrodes (connected to a constant voltage Grass SD9 stimulator) were attached to the right auricle. CO (minus coronary flow) was measured with an electromagnetic flow probe (connected to a Skalar flow meter) placed around the ascending aorta and SV was obtained by integration of the aortic flow curve per beat. A precision occluding device was applied around the aorta just cranial to the diaphragm to permit mechanical variation of cardiac afterload. MAP was measured from a catheter inserted into the left brachial artery. The hemodynamic parameters were recorded on a Grass polygraph and the results were analysed with the aid of an on-line computer system (Hässle hemodynamic system; see Axenborg & Elg 1981). The observed aortic flow values (below called CO) in the control state agreed with data in the literature using the same technique on open-chest cats (cf. Borgdorff 1983, Ordway & Longhurst 1983). Max dP/dt is reported to be very responsive to change in the inotropic state, but relatively insensitive to changes in preload and afterload (Braunwald & Ross 1970), which is in agreement with the findings in the present study (cf. Fig. 8).

Microsphere technique (paper V)

The radioactive microsphere technique (cf. Rudolph & Heymann 1967, Hales 1974, Heymann et al. 1977) was used for observations of changes of regional vascular resistances, the fractional distribution of CO to the tissues, and of CO evoked by hemorrhage on spontaneously breathing animals with intact and blocked β_2 -adrenoceptors. HR, SV, MAP and TPR were also determined in this study.

A polyethylene catheter (o.d. 0.96 mm) for microsphere injections was inserted via the left common carotid artery and its tip was gently advanced into the left cardiac ventricle. Another catheter in the left brachial artery was used for exsanguination and a third catheter in the right brachial artery was used for determination of MAP and HR. Microspheres of $15 \pm 1 \mu\text{m}$ diameter (SD; New England Nuclear, Boston, Mass), with prelabelled specific activity (^{141}Ce or ^{103}Ru of $10 \text{ mCi} \times \text{g}^{-1}$) were used. Arterial reference blood was withdrawn with a pump at a constant rate of $1.74 \text{ ml} \times \text{min}^{-1}$, beginning 15 s before the microsphere injection and continuing for total of 120 s. A first microsphere

injection (^{141}Ce) was made in the control period and a second one (^{103}Ru) was made after experimental intervention. After the second microsphere injection the animals were sacrificed and tissue samples removed. Tissue and reference sample radioactivity was counted (Selectronik 45-22). Injected activity was measured in a well-type ionization chamber (Capintec CRC 4). Calculations of circulatory functions were described in paper IV. There was a minimum of 400 spheres in each tissue sample and a minimum of 2000 spheres in each reference blood sample implying that the counting errors were below 20% at the 95% confidence level (Buckberg et al. 1971, Dole et al. 1982). Adequate mixing of the microspheres in the arterial blood after injections was obtained, as indicated by the finding that the radioactivity in the left and right kidney did not differ by more than 15% in any animal. The effects on CO of bleeding and β_2 -blockade were similar when determined with the microsphere technique (V) and the indicator-dilution technique (II and III), although the magnitude of CO in the resting control state was smaller with the latter technique.

CHEMICAL ANALYSES

Arterial blood glucose (glucose-oxidase method, Boehringer Mannheim), arterial plasma lactate (enzymatically, Boehringer Mannheim), arterial plasma glycerol (enzymatically according to Laurell & Tibbling 1966), and arterial blood pO_2 , pCO_2 , pH, and base excess (IL-413, Instrumentation Laboratories) were determined in paper VI. Arterial plasma adrenaline and noradrenaline concentrations were measured electrochemically according to Eriksson & Persson (1982) in paper IV and VI. Arterial plasma osmolality (thermistor-cryoscopy, Osmometer 31 LAS, Advanced Instruments Inc.) was determined in paper I and VI.

STATISTICS

Spread of data is given as standard error of the mean (SE). Statistical significances were tested according to Student's t-test for independent variables (I-III, VI) and for paired observations (V). In paper IV analysis of variance was used, followed by multiple comparisons according to the Newman-Keuls test (see Miller 1981). P-values <0.05 were considered to be significant.

RESULTS AND COMMENTS

1. ROLE OF β_2 -ADRENERGIC MECHANISMS IN THE CONTROL OF PLASMA VOLUME RESTITUTION IN HEMORRHAGE

Hemorrhage is known to be associated with a considerable net transcapillary absorption of extravascular fluid into the circulatory system, a process mainly occurring in skeletal muscle with its large extravascular fluid reserve. This fluid absorption process is to some extent governed by a glucose-osmotic transcapillary absorption mechanism, but mainly effected via reflex sympatho-adrenal α - as well as β -adrenergic effects directly on the vasculature of skeletal muscle (see Introduction). The resulting net fluid gain into the circulatory system is believed to contribute importantly to posthemorrhagic compensatory plasma volume (PV) restitution.

The aim of the present series of expts was to determine directly the PV restitution after acute hemorrhage and to evaluate the extent to which β_2 -adrenergic control mechanisms might contribute to this process. For this purpose PV restitution after standardized graded hemorrhage was determined in anaesthetized cats in the absence and presence of effective β_2 -adrenoceptor blockade, accomplished by intravenous administration of the highly selective β_2 -adrenoceptor blocking agent ICI 118,551.

Comparative observations were made on 4 groups of animals, viz. on cats with intact and blocked β_2 -adrenoceptors exposed to rapid withdrawal of 10 or 20 ml of blood $\times$ kg bwt⁻¹, corresponding to about 20 and 40% reductions of total blood volume. Data on PV, arterial hematocrit, and estimated blood volume were obtained in the control period prior to, and at 1 and 2 h after, bleeding. The prehemorrhagic control data did not differ significantly in the 4 groups (Table 1, paper I): PV averaged about 34 ml $\times$ kg bwt⁻¹, blood volume about 50 ml $\times$ kg bwt⁻¹, and arterial hematocrit about 34%. These data are in agreement with previous reports on cats (see Methods section).

Fig. 1 shows the extent of PV restitution (ml $\times$ kg bwt⁻¹) in cats with intact and blocked β_2 -adrenoceptors as determined 1 and 2 h after the initial reduction of blood volume by 10 and 20 ml $\times$ kg bwt⁻¹. With an observed hematocrit value of 34% this corresponded to an initial decrease

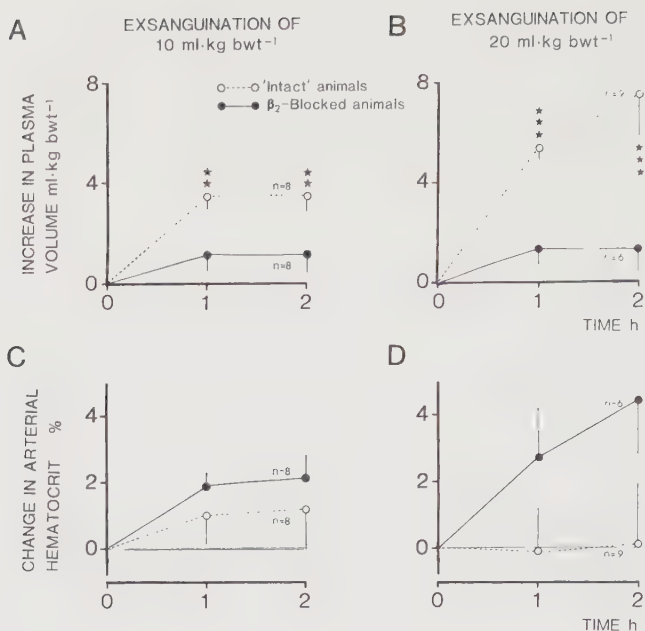


Fig. 1. Restitution of plasma volume (panels A and B) and changes of arterial hematocrit (panels C and D) after acute graded hemorrhage in animals with intact (open circles) and systemically blocked (closed circles) β_2 -adrenoceptors. ** p<0.01; *** p<0.001.

in PV by 6.6 and 13.2 ml $\times$ kg bwt⁻¹, respectively. It can be seen from panels A and B that PV increased in both the intact and the β_2 -blocked animals after bleeding. The increase was, however, significantly greater in the former animals. With intact β_2 -adrenoceptors, the PV restitution was 3.4 ml $\times$ kg bwt⁻¹, or 52% of the shed PV, at both 1 and 2 h after the smaller blood loss (panel A), and 5.4 and 7.5 ml $\times$ kg bwt⁻¹, or 41 and 57%, at 1 and 2 h after the more pronounced bleeding (panel B). After β_2 -blockade, the PV restitution was only 1.1 and 1.4 ml $\times$ kg bwt⁻¹, or 17 and 11% of the shed PV, at these two degrees of bleeding. These data indicate that β_2 -adrenergic control mechanisms are of great quantitative importance for the compensatory PV restitution after blood loss.

The changes of arterial hematocrit observed in response to hemorrhage are shown in the lower panels of Fig. 1. In cats with intact β_2 -adrenoceptors, hematocrit tended to increase somewhat in response to the smaller blood loss (panel C), but was unchanged after the more pronounced bleeding (panel D). In the animals with blocked β_2 -adrenoceptors there was a significant gradual increase in hematocrit at both levels of

bleeding, corresponding to 2.0 and 4.4% 2 h after exsanguination. The fact that hematocrit remained unchanged or even increased in the phase of PV restitution indicates a concomitant increase in circulating red cell volume. This effect was mainly caused by recruitment of red cells from the spleen contracting in response to hemorrhage, as evidenced by the finding that, after acute splenectomy, bleeding ($10 \text{ ml} \times \text{kg}^{-1}$) caused a 7% decrease in hematocrit in cats with intact adrenoceptors.

COMMENTS

These results demonstrated an efficient compensatory control of PV after acute hemorrhage, which within 1-2 h corresponded to restitution of more than 50% of the initially lost PV (or about 35% of the shed blood volume). The major part (some 75%) of this PV restitution could be ascribed to a β -adrenergic control mechanism. Hillman (1983) studied the rates of transcapillary fluid absorption in the lower leg muscle of the cat in hemorrhage in the presence and absence of regional β -blockade and found that this process also to a greater part (about 70%) could be attributed to a β -adrenergic control mechanism. The quantitative importance of such cumulative net transcapillary fluid absorption for PV restitution in bleeding was estimated by Hillman (1983) by extrapolation of the data for the observed net fluid gain from the studied muscle region to the entire skeletal muscle mass of the cat. Such extrapolated figures were of an order of magnitude that fully could account for the directly determined PV expansion observed in this study. These data, taken together, are therefore compatible with the view that the main mobile fluid reservoir for PV replenishment in bleeding is that present in the extravascular space of skeletal muscle, in agreement with other reports (e.g. Öberg 1964, Eliassen et al. 1974, Järhult 1975, Reed & Wiig 1981).

The posthemorrhage PV replacement remaining after β_2 -adrenoceptor blockade (Fig. 1) most likely is explained by fluid absorption from muscle caused by the α -adrenergic and glucose-osmotic mechanisms mentioned in Introduction. The β_2 -adrenergic fluid absorption in skeletal muscle was attributed to a fall in capillary hydrostatic pressure evoked by a preferential β_2 -adrenergic dilator action on the postcapillary resistance vessels and to an increase in the functional capillary surface area evoked by β -adrenergic relaxation of precapillary 'sphincters' (see Hillman 1983). Other possible β_2 -adrenergic mechanisms contributing to

the different rates of net fluid absorption in muscle in the absence and presence of β -adrenergic blockade, such as a rheological effect, interference with capillary permeability, or interference with peripheral autonomic nervous activity by β -blockade, seemed to be ruled out as discussed by Hillman (1983).

In the above described investigations the β -blockade was limited to the studied skeletal muscle region to avoid systemic effects. However, in the present expts the β_2 -blocking agent ICI 118,551 was given intravenously and such systemic blockade might have influenced other than vascular β_2 -adrenergic mechanism. For instance, it might have blocked possible fluid gain to the circulation from other sites than skeletal muscle. Sympatho-adrenal β -receptor activation of the juxta-glomerular apparatus in the kidney is known to cause release of renin and, hence, aldosterone, but in the cat this effect is claimed to be mediated via β_1 -adrenoceptors (cf. Keeton & Campbell 1980, Johns 1981), implying that it would not have been interfered with in the present expts by the selective β_2 -adrenoceptor blocking agent. Fluid gain to the circulation might also occur from the small intestine during hemorrhage, apparently mediated by sympatho-adrenal activation (Redfors 1983, Redfors & Sjövall 1984). There is, however, as yet no evidence for involvement of β_2 -adrenoceptors in this effect. As stated by Redfors the intestinal fluid gain might require that considerable amounts of fluid are present in the intestinal lumen, which probably was not the case in the present expts performed on animals starved for about 15 hours before the expt. β_2 -adrenoceptors are known to be present in the central nervous system but their function is poorly defined (Middlemiss, Buxton & Greenwood 1981, Minneman, Pittman & Molinoff 1981). Since ICI 118,551 is lipid soluble it probably has access to these receptors, but no evidence of depressed central or peripheral nervous autonomic activity was revealed, for instance in terms of a less pronounced reflex increase in HR in response to bleeding in the β_2 -adrenoceptor blocked cats (see below, sections 2 and 5). Some β_2 -adrenoceptor blocking agents are reported to enhance platelet aggregation (Winther, Knudsen & Gormsen 1983) which, in turn, might affect blood rheology, especially in postcapillary vessels. This might apply also to compound ICI 118,551, but probably not in the low dose used in the present study (personal communication from the manufacturer).

Finally, the possibility that β_2 -adrenergic effects on vascular

permeability might have affected the present results will be considered. It is known that agents like histamine, bradykinin, substance P, etc., as well as induced anaphylactic reactions, can increase vascular permeability to macromolecules for periods of 15-30 min (for ref. see Persson & Svensjö 1982). Exogenous administration and endogenous release of catecholamines can attenuate such increases in macromolecular permeability and this effect seems to be mediated via activation of β_2 -adrenoceptors (Marciniak et al. 1977, Svensjö, Persson & Rutili 1977, O'Donnell & Persson 1978, Dobbins et al. 1982). There is, however, no evidence at present for increased macromolecular permeability in hemorrhagic hypovolemia and therefore no reason to believe that the observed β_2 -adrenergic control of PV should be related to effects on macromolecular (protein) exchange between the intravascular and extravascular space. Further, as mentioned above, strong experimental evidence indicates that the β_2 -adrenergic PV control more or less completely can be explained by β_2 -adrenergic regulation of capillary hydrostatic pressure and of precapillary sphincter tone in skeletal muscle.

The main conclusion from the present series of expts is that there is an important β_2 -adrenergic control of PV restitution in bleeding. It is quite likely that such volume expansion in hemorrhage can have beneficial effects on central hemodynamics, a problem approached in the next section of Results.

2. INFLUENCES OF β_2 -ADRENERGIC VASCULAR CONTROL MECHANISMS ON CENTRAL HEMODYNAMICS IN HEMORRHAGE

From the results presented in the previous section it was established that the compensatory PV restitution after hemorrhage was significantly greater in animals with intact than with selectively blocked β_2 -adrenoceptors. Such β_2 -adrenergic control of PV would be expected to improve venous return and cardiac filling during hemorrhage. It is also possible that a β_2 -adrenergic dilator interaction with constrictor mechanisms on arterial vascular tone during hemorrhage might affect TPR. Peripheral β_2 -adrenergic effects might thus influence central hemodynamics during hemorrhage, a problem approached in this series of expts by analysing effects on CO, HR, SV, MAP, and TPR during standardized bleeding in animals with intact and selectively blocked β_2 -adrenoceptors.

Central hemodynamic response patterns to standardized hemorrhage

Bleeding in a first series of expts was accomplished by a standardized rapid (<3 min) withdrawal of 20 ml of blood $\times$ kg bwt⁻¹ corresponding to an about 40% decrease in the cat's total blood volume.

Table 1. Control values for central hemodynamic variables in cats prior to bleeding.

	MAP (mmHg)	CO (ml·min ⁻¹ ·kg ⁻¹)	TPR (mmHg·min·kg·ml ⁻¹)	HR (beats·min ⁻¹)	SV (ml·kg ⁻¹)
Intact animals n=12	124±5	129±5	0.99±0.06	211±11	0.63±0.04
β_2 -blocked animals n=13	122±6	132±8	0.96±0.07	198±7	0.68±0.05

Table 1 (taken from the material in paper III) shows the observed mean values $\pm$ SE in the control period prior to hemorrhage for the mentioned central hemodynamic variables in animals with intact and selectively blocked β_2 -adrenoceptors. These control values were not significantly different in the two groups of animals indicating that the β_2 -adrenergic influence on the circulation in the resting control state was small.

Fig. 2 shows the collected data (paper III) on the changes from the control state (Table 1) of the central hemodynamic variables evoked by the standardized hemorrhage. The data refer to observations during a 2 h period after bleeding.

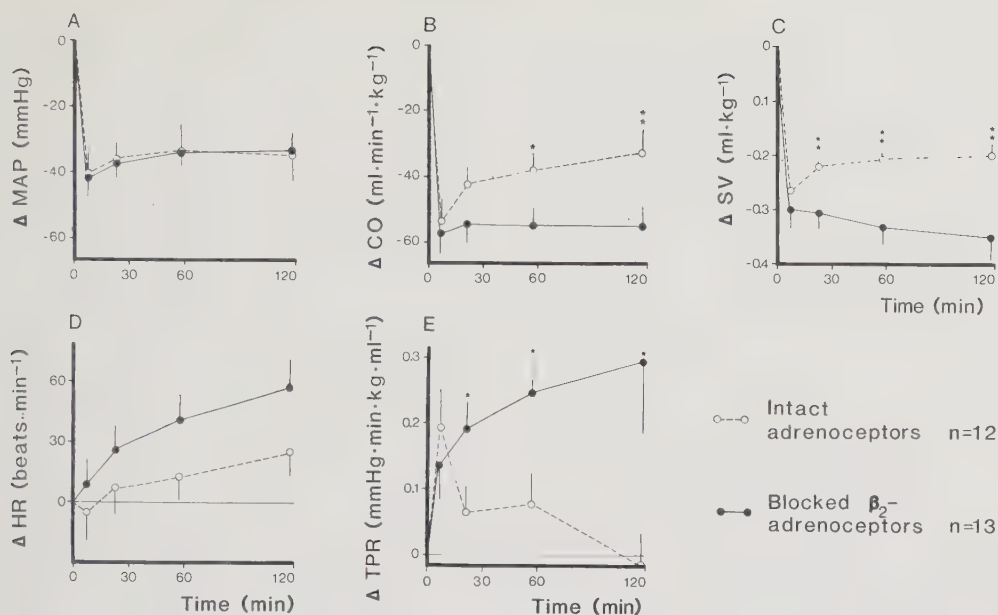


Fig. 2. Diagrams showing changes of mean arterial blood pressure (MAP), cardiac output (CO), stroke volume (SV), heart rate (HR), and total peripheral resistance (TPR) after acute exsanguination of 20 ml $\times$ kg bwt^{-1} in animals with intact and systemically blocked β_2 -adrenoceptors. * $p < 0.05$; ** $p < 0.01$.

Just after completion of the bleeding procedure, MAP was decreased on the average by 75–80 mmHg below the control value in both groups of animals and then showed a quick recovery (not included in Fig. 2). The first set of data in the figure refer to the average time period for the first triplicate cardiac output determinations (7 min after hemorrhage). At this time, MAP was about 40 mmHg below control (Fig. 2, Panel A) and showed a slight further recovery during the remainder of the observation period. The MAP response was not significantly different in animals with intact and blocked β_2 -adrenoceptors.

Other central hemodynamic variables, however, differed markedly in the two groups of animals during the 2 h observation period after bleeding (except for the very early events). CO (Panel B) decreased by about 55 $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$ (or by about 45%) 7 min after hemorrhage in both groups of cats; later on, there was a clear-cut partial recovery of CO in the intact but not in the β_2 -blocked animals. Thus, 2 h after bleeding, CO was almost 25 $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$ larger in the intact than in the β_2 -blocked cats. The HR and SV responses underlying these CO adjustments are shown in Panels C and D. SV first decreased by a similar

amount in both groups, later on followed by a partial recovery in the animals with intact β_2 -adrenoceptors, but by a gradual deterioration of SV in the β_2 -blocked cats. Immediately upon bleeding there was a transient tachycardia response in both groups (not shown in Fig. 2). Later on, a moderate compensatory tachycardia developed gradually which appeared to be more pronounced in the β_2 -blocked animals even if the spread in the material was such that no significant difference between the two groups could be established. The partial posthemorrhagic recovery of CO in animals with intact β_2 -adrenoceptors apparently can be attributed to increased SV as well as HR, whereas in the β_2 -blocked cats the tachycardia barely helped to maintain CO at the initial low level in the face of a gradually declining SV. TPR (panel E) increased initially (at 7 min) to about the same extent in both groups. Subsequently, there was a gradual restoration of TPR in the animals with intact adrenoceptors, returning to the control level about 2 h after bleeding, whereas TPR continued to increase throughout the observation period in the β_2 -blocked cats. Thus, 2 h after exsanguination, TPR was about 30% higher in animals with blocked than with intact β_2 -adrenoceptors.

It may be concluded that cats with intact and blocked β_2 -adrenoceptors, exposed to the same initial exsanguination, during the subsequent 2 h period establish a similar MAP level, though by different mechanisms. In the animals with intact adrenoceptors there was a gradual partial recovery of SV and CO in the face of a gradual restoration to control of an initially raised TPR. In the animals with blocked β_2 -adrenoceptors a gradual further rise in peripheral resistance instead occurred in the face of maintained low CO and declining SV.

Patterns of response to graded hemorrhage

The central hemodynamic patterns of response to graded hemorrhage, studied on animals with intact and selectively blocked β_2 -adrenoceptors, are illustrated in Fig. 3. The data refer to observations of MAP, CO, SV, HR, and TPR during a 2 h period after acute exsanguination of 5, 10, and 20 ml $\times$ kg⁻¹ (corresponding to about 10, 20, and 40% of the total blood volume). The data for the largest bleeding (the panels to the right) are reproduced from Fig. 2 to facilitate direct comparison of the results. In this summary it may suffice to describe only in general terms these patterns of response (for details see paper III).

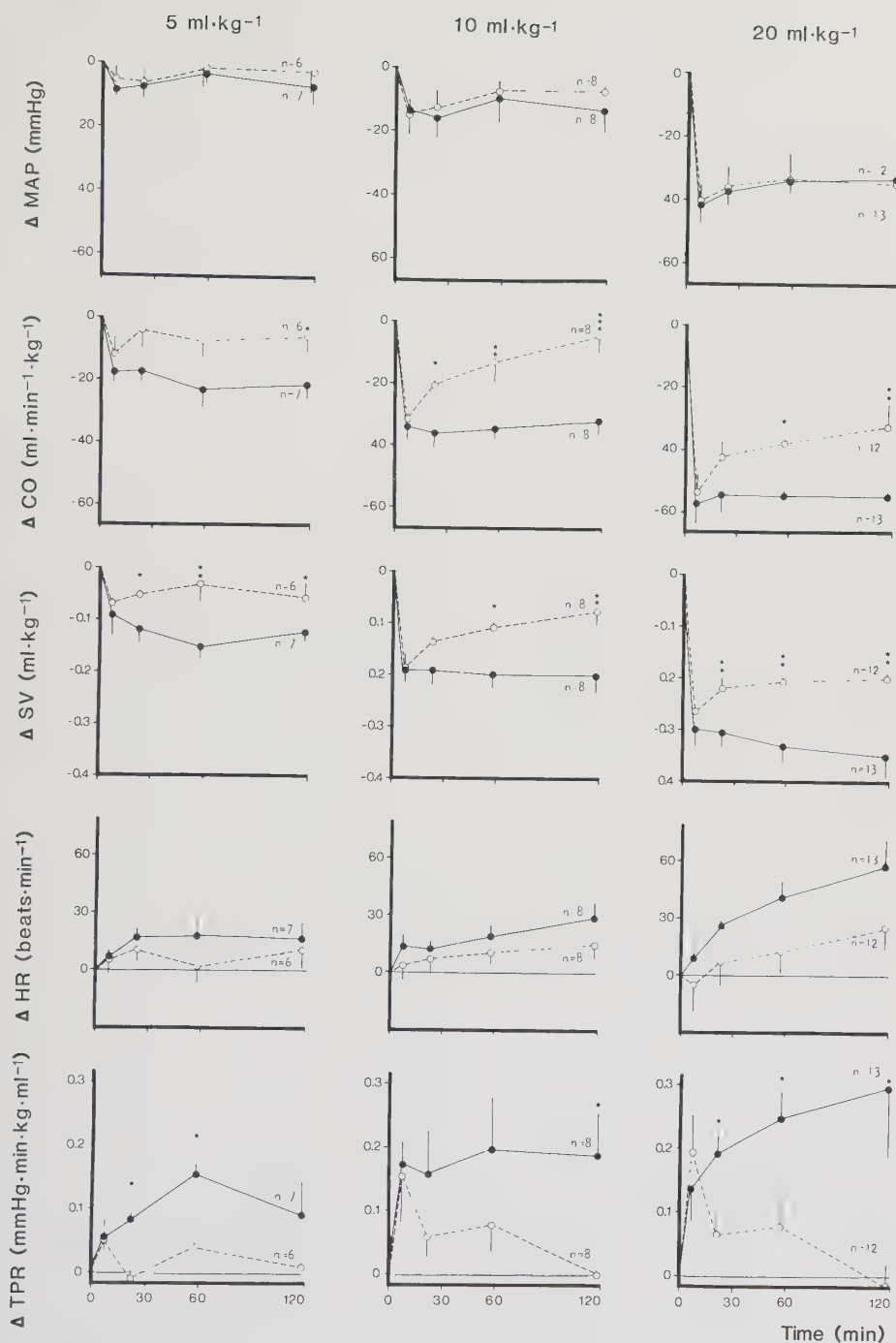


Fig. 3. Changes of central hemodynamic variables after acute graded hemorrhage (exsanguination of 5, 10, and 20 ml x kg⁻¹) in animals with intact (open circles) and systemically blocked (closed circles) β_2 -adrenoceptors. * p<0.05; ** p<0.01; *** p<0.001.

It can be seen that the central hemodynamic response patterns in animals with intact and blocked β_2 -adrenoceptors evoked by exsanguination of 5 and 10 ml $\times$ kg⁻¹ (left panels) were qualitatively quite similar to the ones observed after withdrawal of 20 ml $\times$ kg⁻¹ of blood. However, the responses were quantitatively different, and roughly graded in relation to the extent of the induced hypovolemia. Such graded effector responses are expected to be evoked by a true physiological regulatory system, and this criterion thus seems to be fulfilled also with regard to the effects elicited by the β_2 -adrenergic control mechanism.

Relation between PV and SV in hemorrhage

The much more effective posthemorrhagic PV restitution observed in animals with intact than with blocked β_2 -adrenoceptors (section 1) might, via improved circulatory and cardiac filling, be a major cause of the SV recovery in the animals with intact adrenoceptors (Fig. 3). This assumption was corroborated by results presented in paper III (Fig. 7), which indicated a roughly direct relation between observed gradual recovery of PV and of SV during the 2 h posthemorrhagic period on animals with intact adrenoceptors. Further support for such a SV dependency of PV was obtained by a comparative analysis on animals with intact and blocked β_2 -adrenoceptors described in paper II. It was found that animals with intact adrenoceptors, exposed to a 40% bleeding, and β_2 -blocked animals, exposed to a 20% bleeding, 2 h later exhibited a roughly equal hypovolemia, mainly due to the more effective PV restitution in the animals with intact adrenoceptors. Under these circumstances both groups of animals exhibited a SV decline which was of the same order of magnitude (paper II, Fig. 3). The tachycardia response and the CO decline were also similar in these two groups. Yet, TPR in the β_2 -blocked animals, in contrast to those with intact adrenoceptors, was maintained clearly above the control value and, as a consequence, the decrease in MAP was more pronounced in the latter than in the former. The cause of the latter discrepancy was subject to analysis in the expts described below.

Influence of vascular β_2 -adrenergic mechanisms on the control of TPR in hemorrhage

The demonstrated fact (section 1) that the compensatory PV restitution in response to a given acute hypovolemia was much more effective with time in animals with intact than with blocked β_2 -adrenoceptors could imply

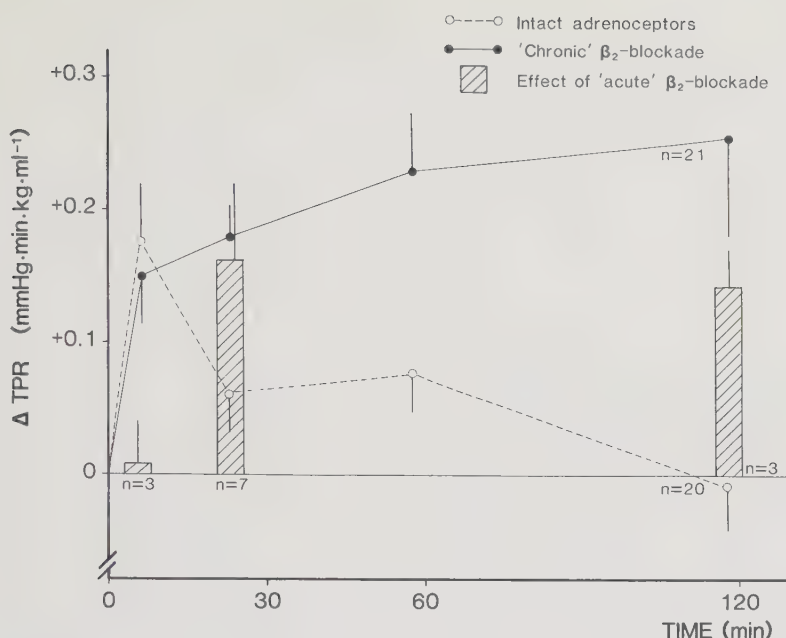


Fig. 4. Hatched bars: Increase in total peripheral resistance (TPR) evoked by 'acute' i.v. β_2 -adrenoceptor blockade instituted at various times (about 5, 25, and 120 min) after hemorrhage (15 ml $\times$ kg bwt⁻¹). Curves: Changes of TPR evoked by the same degree of bleeding in animals in which the adrenoceptors were intact throughout the 2 h observation period (open circles) and in which the β_2 -adrenoceptors were selectively blocked prior to hemorrhage ('chronic' β_2 -blockade, closed circles).

that the mechanoreceptor induced reflex sympatho-adrenal activity gradually declines, especially in animals with preserved β_2 -adrenergic PV control. Such an effect, in turn, might explain the observed gradual posthemorrhagic decline in TPR in the intact animals contrasting to the maintained high TPR in the β_2 -blocked animals (Fig. 3). An alternative explanation could be that, on the animals with intact adrenoceptors, TPR declines as a result of a β_2 -adrenergic dilator interaction with constrictor influences on the tone of the systemic resistance vessels.

The latter possibility was examined experimentally by investigating on exsanguinated animals with intact adrenoceptors the effect on TPR of selective β_2 -adrenergic blockade instituted acutely at various times (about 5, 25, and 120 min) after bleeding (Fig. 4, hatched bars). Pilot experiments (e.g. paper III, Fig. 1) had demonstrated that effective β_2 -adrenergic blockade is obtained very quickly (within less than 3 min) after i.v. administration of compound ICI 118,551. In Fig. 4 are also inserted, for comparison, curves depicting the TPR changes in response to the same degree of bleeding on animals in which the adrenoceptors were

intact throughout the 2 h observation period (open circles) and in which the β_2 -adrenoceptors were selectively blocked prior to the hemorrhage ('chronic blockade', closed circles).

It can be seen from Fig. 4 (hatched bars) that acute β_2 -blockade instituted 25 and 120 min after bleeding evoked a significant quick rise in TPR. At 25 min, this resistance effect of acute β_2 -blockade, if anything, was larger than that noted after chronic blockade (cf. difference between closed and open circles in the two curves); at 120 min the effect of acute blockade was about 55% of that observed with chronic blockade. These data, taken together, are interpreted to signify the presence of a β_2 -adrenergic dilator interaction with constrictor influences on the tone of the systemic resistance vessels on hypovolemic animals with intact adrenoceptors, most likely mainly evoked by adrenaline released from the adrenals. Such release in significant amounts seemed to occur only gradually during hemorrhage, as suggested by the relatively small TPR effect of acute β_2 -blockade observed early (5 min) after bleeding (Fig. 4).

Fig. 5 shows the central hemodynamic response pattern to acute β_2 -blockade, in this case instituted 25 min after bleeding. The data are expressed as percent changes from the control values immediately before blockade. The acute blockade caused a 17% increase in TPR due to an abrupt rise of MAP and a concomitant decrease in CO, the latter explained by slight decreases in both HR and SV. It is likely that an effect of β_2 -adrenergic blockade directly on the vascular smooth muscle (via raised vascular resistance) is the primary event explaining the rise in TPR and MAP, and that the cardiac effects are secondary to the MAP response, for instance via increased cardiac afterload. A negative chronotropic effect of β_2 -blockade may, however, not be entirely ruled out. These possibilities will be explored in Results, section 3.

The described results thus may permit the conclusion that there is a pronounced β_2 -adrenergic dilator interaction with the constrictor influences on the tone of the systemic resistance vessels in hemorrhage, an effect apparently contributing significantly to the observed gradual restoration to control of the initially raised resistance in animals with intact adrenoceptors. However, it is evident that other factors, such as reflexly decreased vasoconstrictor influence as a result of the gradual

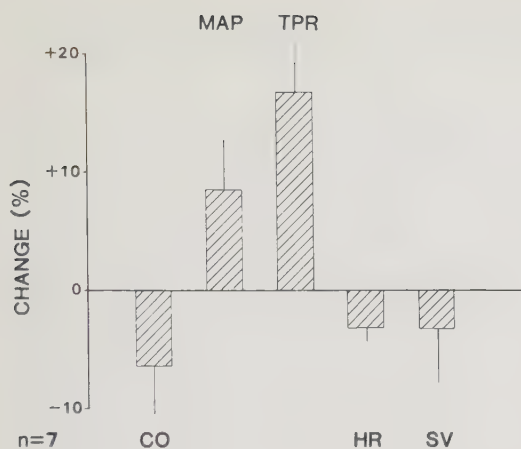


Fig. 5. Effects on CO, MAP, TPR, HR, and SV evoked by 'acute' i.v. β_2 -adrenoceptor blockade instituted about 25 min after hemorrhage (15 ml $\times$ kg bwt⁻¹).

plasma volume restitution, also might contribute with time to the systemic resistance decline.

COMMENTS

Although the literature about circulatory effects of nonselective and 'cardioselective' β -adrenoceptor blocking agents is abundant, there is, to our knowledge, no previous study in which a selective β_2 -adrenergic blocking agent has been employed for the study of the central hemodynamics during reflex sympatho-adrenal activation. This study showed that cats with intact and selectively blocked β_2 -adrenoceptors, exposed to the same initial exsanguination, established similar levels of maintained arterial hypotension during the 2 h observation period. Other central hemodynamic variables, however, differed markedly in the two groups of animals. In cats with intact adrenoceptors there was a gradual partial recovery of SV and CO in the face of a gradual restoration to control of an initially raised TPR. In contrast, the β_2 -blocked animals showed a gradual further rise of peripheral resistance in the face of a maintained low SV and CO. These different patterns of response after a small ($\sim 10\%$), moderate ($\sim 20\%$) and large ($\sim 40\%$) blood loss were qualitatively similar, but the magnitude of the β_2 -adrenergic effects were directly related to the magnitude of the hypovolemia. The observed recovery of SV and CO in animals with intact β_2 -adrenoceptors most likely can be ascribed to indirect effects on cardiac performance, especially to improved cardiac filling resulting from the β_2 -adrenergically controlled PV restitution in bleeding (section 1). The gradual decline in TPR in animals with intact adrenoceptors apparently was caused mainly by a β_2 -adrenergic dilator interaction with constrictor

influences on the systemic resistance vessels. These interpretations deserve some further comments.

The comparison (Figs 4 and 5) of the TPR effects observed in the absence and presence of chronic β_2 -blockade (applied before bleeding) and in response to acute β_2 -blockade instituted at various times after bleeding provided evidence for a direct β_2 -adrenergic dilator interaction with constrictor influences in hemorrhage on the tone of the systemic resistance vessels in animals with intact adrenoceptors. This interpretation is supported by our previous findings of such a dilator interaction in several hemodynamically important vascular areas (skeletal muscle, intestine, skin, and to some extent the kidneys) revealed by instituting strictly regional β -blockade (cf. Hillman & Lundvall 1980, Lundvall & Gustafsson 1981). With this experimental approach, the observed β_2 -adrenergic alteration of resistance must represent a direct effect on the vascular smooth muscle, since possible indirect effects of the blockade on the heart and/or central autonomic structures were avoided. Evidence of a β -adrenergic dilator component in the control of vascular resistance in skeletal muscle has also been reported during reflex sympatho-adrenal activation by other means than hemorrhage, viz. by hypoxia (Chalmers, Korner & White 1966).

It is possible, however, that some other factors than the β_2 -adrenergic dilator mechanism could have contributed to the observed difference in the TPR responses to hemorrhage in the animals with intact and blocked β_2 -adrenoceptors. Mention has been made that the more pronounced PV restitution and, hence, increased circulatory filling in the former group of animals quite likely might have led to a gradual decline of the sympathetic discharge rate in the posthemorrhagic period (cf. Fig. 4). The greater PV restitution in the animals with intact adrenoceptors further tended to lower arterial hematocrit (cf. Figs 1 and 10) and thereby blood viscosity to a greater extent than in the animals with blocked β_2 -adrenoceptors. It has been shown that a decrease in hematocrit in bleeding, if pronounced, can mask an active increase in vascular resistance by its passive rheological effect (Chien et al. 1973). However, the difference in hematocrit observed in our study between animals with intact and blocked β_2 -adrenoceptors was much smaller than the hematocrit alterations studied in the expts of Chien et al. It is therefore believed that the hematocrit induced rheological influence on

TPR in the cats with intact and blocked β_2 -adrenoceptors in the present study can be considered roughly comparable and small. An effect on TPR of lower shear rate in the latter group due to lower peripheral blood flows and possibly lower blood flow velocities might, however, not be entirely excluded.

The noradrenaline release from sympathetic nerve endings is known to be increased to some extent via presynaptic β -adrenoceptor stimulation. From *in vitro* studies it has been claimed that these receptors are of the β_2 -subtype (cf. Langer 1980). *In vivo* observations, however, clearly indicate that they are of the β_1 -subtype, at least in the vascular bed of cat skeletal muscle (Dahlöf 1981), implying that this mechanism would not be interfered with by compound ICI 118,551. If in some tissues presynaptic β_2 -adrenoceptors do exist, blockade of these by the drug would, if anything, have led to an underestimation of the described TPR difference between animals with intact and blocked β_2 -adrenoceptors.

It might be argued that the recovery of SV and CO during bleeding in animals with intact adrenoceptors might not merely be due to the β_2 -adrenergic restitution of PV and thereby improved cardiac filling, but also to an effect on venous return via an influence on vascular capacitance. Even if the direct β -adrenergic influence is known to cause slight dilatation, if anything, of the capacitance vessels (e.g. Johnsson & Öberg 1968, Greenway 1981, Lundvall et al. 1982) and hence decreased venous return, a redistribution of blood from the systemic to the pulmonary circulation was observed in response to isoprenaline infusions in cats and dogs (Green 1977, Imai, Satoh & Taira 1978, Greenway 1979, Rutlen, Supple & Powell 1981). In the dog, this effect was attributed to a β -adrenergic relaxation of postsinusoidal hepatic sphincters (Rutlen et al. 1981) and in the cat it was ascribed to a renal β -adrenergic release of renin-angiotensin with a postulated selective constrictor effect on the splanchnic capacitance vessels (Greenway 1979). In the present expts, the net effect of β_2 -adrenergic control mechanisms on the capacitance vessels seemed to be small, as suggested by observations (to be described in section 3) of quite similar effects on central venous pressure and left ventricular preload (LVEDP) of brief infusions of the endogenous β -adrenoceptor agonist, adrenaline, on cats with intact and blocked β_2 -adrenoceptors. The possible existence of β_2 -adrenergic chronotropic and inotropic effects on the heart will also be explored in section 3.

3. CENTRAL HEMODYNAMIC EFFECTS OF ADRENALINE WITH SPECIAL REFERENCE TO β_2 -ADRENERGIC INFLUENCES ON HEART RATE AND CARDIAC AFTERLOAD

The results presented in section 2 indicated that a β_2 -adrenergic dilator interaction with the constrictor influences on the systemic resistance vessels to a significant extent was responsible for the gradual restoration to control of an initially raised TPR in bleeding. Such a β_2 -adrenergic dilator influence might via lowered cardiac afterload have a beneficial influence on CO. This problem was approached in paper IV by a 'model' study in normovolemic cats of the β_2 -adrenergic effects of i.v. administered adrenaline and noradrenaline on TPR and cardiac afterload and performance. Attempts were also made to reveal possible direct β_2 -adrenergic cardiac effects resulting from the postulated co-existence of β_2 - and β_1 -adrenoceptors in the heart (Carlsson et al. 1972).

The integrated net cardiovascular effects of α - and β -adrenoceptor activation by the endogenous catecholamines have been studied extensively (for ref. see Weiner 1980, Greenway 1981). The influences of nonselective β -blockade and of 'cardioselective' β_1 -blockade on these responses are also well documented (e.g. Shanks 1966, Brick et al. 1968, Dunlop & Shanks 1968, Åblad et al. 1974, Johnsson 1975, Herwaarden et al. 1977), and may provide some qualitative information about the β_2 -adrenergic component in the response. However, more specific and reliable information about such effects would be obtained by the present approach using a selective β_2 -blocking agent, especially with regard to indirect effects on cardiac performance via changed afterload.

The expts were performed on closed-chest cats (observations of CO, HR, SV, TPR, and MAP) as well as on open-chest cats (observations of CO, HR, SV, TPR, MAP, LVEDP, max dP/dt, CVP, and LAP). Adrenaline and noradrenaline were infused in doses causing graded plasma concentrations from 3 to 120 nM which covers the range encountered in bleeding (cf. Watts 1965, Jakschik et al. 1974, Farnebo et al. 1979, Hillman 1981, Andersson et al. 1982). Comparative observations were made on animals with intact adrenoceptors, after selective β_2 -blockade (ICI 118,551), and after nonselective β -blockade (propranolol).

Control data prior to catecholamine infusion

The pre-infusion control values for the abovementioned central

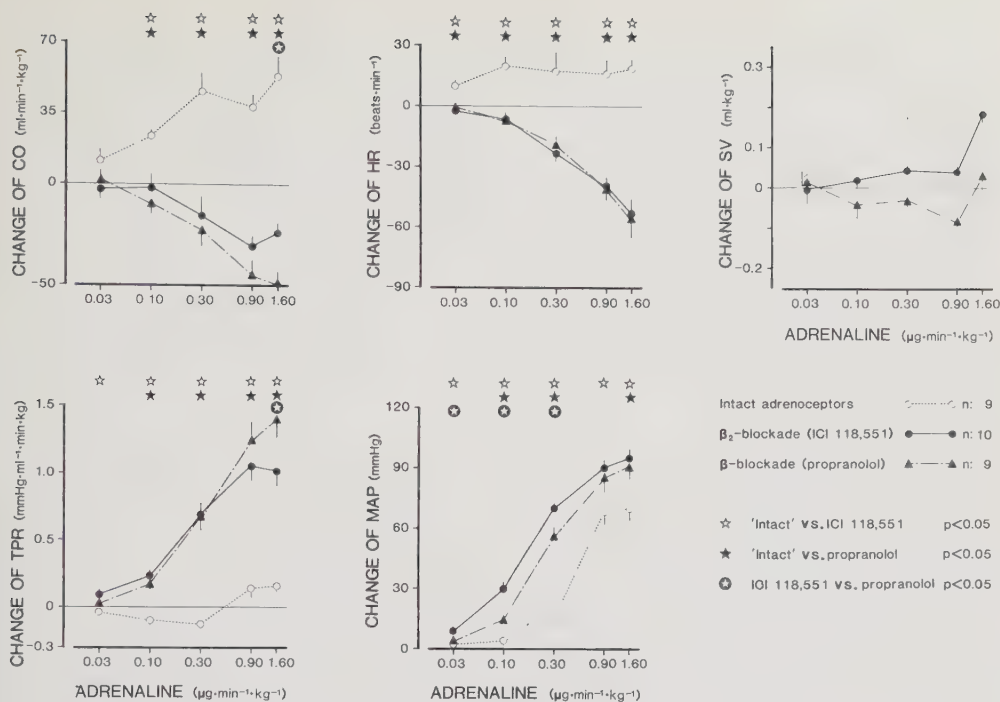


Fig. 6. Diagrams showing changes of central hemodynamic variables evoked by i.v. infusion of adrenaline in graded doses on closed-chest animals with intact vagal nerves. Observations were made on animals with intact adrenoceptors (open circles), after selective β_2 -adrenoceptor blockade (closed circles), and after nonselective β -blockade (closed triangles). ☆, ★, ⊕ indicate $p < 0.05$, see key.

hemodynamic variables (paper IV, Table 1) were not significantly altered by selective β_2 -blockade. Combined β_1 - and β_2 -blockade by propranolol, however, caused significant negative chronotropic and inotropic responses.

Effects of adrenaline in closed-chest cats with intact vagal nerves

Fig. 6 shows the changes in the central hemodynamic variables in the closed-chest cats with intact vagal nerves evoked by i.v. infusions of adrenaline. With intact adrenoceptors (open circles), adrenaline caused a roughly dose dependent increase in CO due to a rise of both HR and SV. TPR decreased in the dose range 0.03-0.30 $\mu\text{g} \times \text{min}^{-1} \times \text{kg bwt}^{-1}$, but this net vasodilator response was converted to net constriction at the higher doses. MAP was largely unchanged at low doses but rose markedly at high doses. After blockade of the β_2 -adrenoceptors (closed circles), CO was unchanged at low doses of adrenaline but decreased gradually at the higher doses due to declining HR. In contrast to the condition with intact β -adrenoceptors, the TPR response to adrenaline after β_2 -blockade showed a pronounced gradual increase, which was responsible for the

concomitant marked increase in MAP. After nonselective β -blockade (closed triangles), finally, the pattern of response to adrenaline was not much different from that observed after selective β_2 -blockade. CO and SV, however, tended to decrease further with a consequent, somewhat less pronounced increase in MAP. It can be concluded from the results in Fig. 6 that the very pronounced adrenaline induced increase in TPR observed after selective β_2 -blockade, and also after nonselective β -blockade, can be ascribed to a dose-dependent, mainly α -adrenergic, vasoconstrictor response, becoming unmasked by the elimination of a β_2 -adrenergic vasodilator influence.

Effects of adrenaline in open-chest vagotomized cats

The open-chest preparation on vagotomized animals was used to analyse in more detail possible adrenaline evoked β_2 -adrenergic effects on cardiac performance (Fig. 7). The central hemodynamic response pattern to two doses of adrenaline (0.1 and $0.3 \mu\text{g} \times \text{min}^{-1} \times \text{kg bwt}^{-1}$, i.v.) was first studied with intact adrenoceptors (open bars), then after β_2 -blockade (hatched bars), and, finally, after nonselective β -blockade (black bars). In the β_2 -blocked preparation gradual occlusions of the descendent aorta were also performed (dotted bars) so as to accomplish increases in cardiac afterload (MAP) roughly similar to those observed during adrenaline infusions after β_2 -blockade. Observations on cats with intact and selectively blocked β_2 -adrenoceptors were performed both in the absence and presence of cardiac pacing ($250 \text{ beats} \times \text{min}^{-1}$) to facilitate the evaluation, especially of the inotropic response (max dP/dt).

With intact adrenoceptors (Fig. 7, open bars) the pattern of response to adrenaline was qualitatively similar to that observed in the closed-chest animals (Fig. 6), i.e. it caused increases in CO, HR, and SV, a decrease in TPR, and a small increase in MAP. LVEDP ('preload') and CVP showed minor increases, whereas max dP/dt (inotropy) rose markedly. The results were basically similar in the absence and presence of cardiac pacing. With blocked β_2 -adrenoceptors (hatched bars) the increases in CO and SV to adrenaline were much smaller (statistically significant) than with intact ones, both in the absence and presence of pacing. This could not be ascribed to differences in preload (LVEDP) or inotropy (max dP/dt) since these parameters, if anything, were greater in the β_2 -blocked preparation (see results during cardiac pacing, Fig. 7). Aortic occlusion per se (dotted bars), causing roughly similar increases in afterload

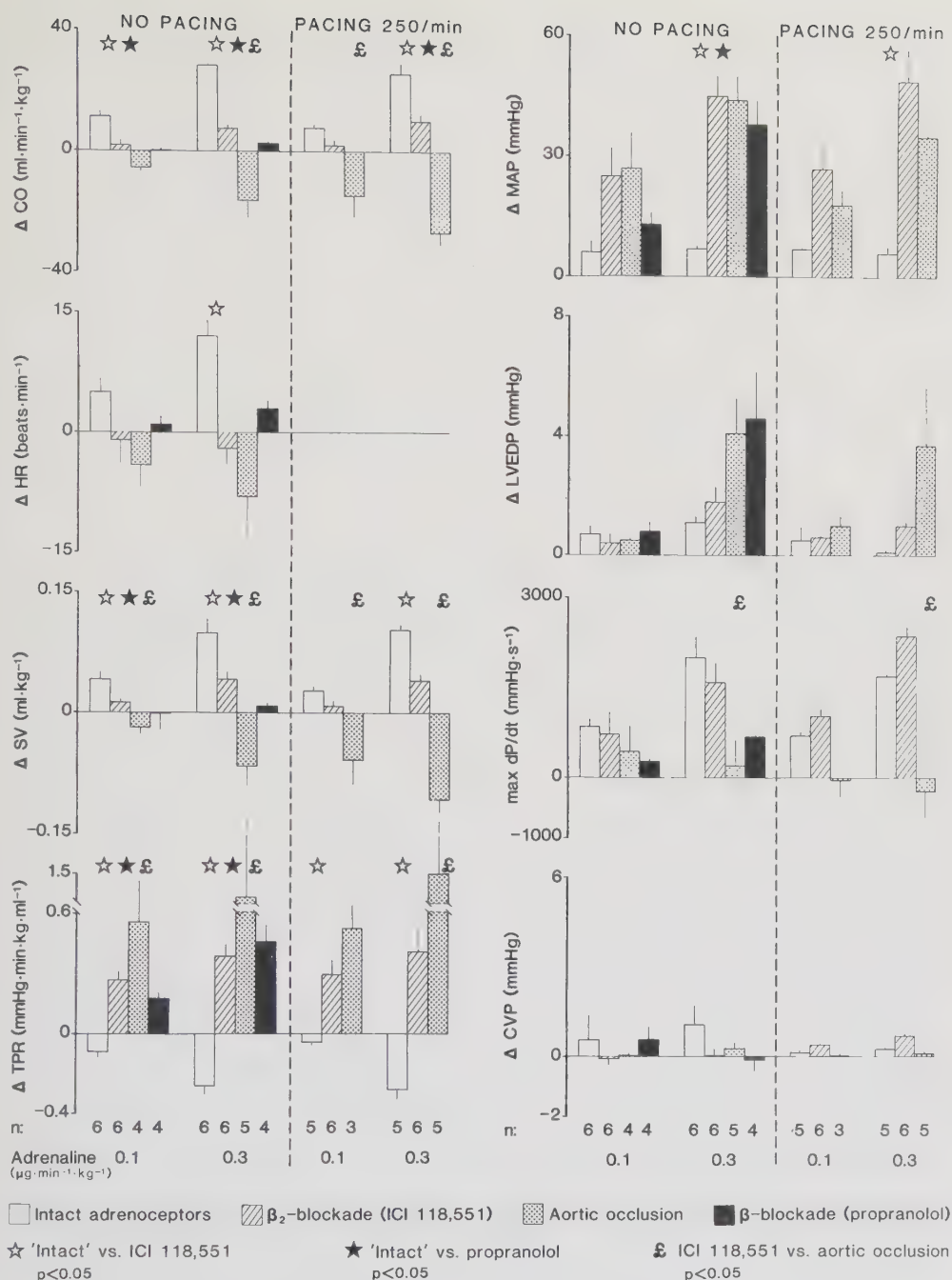


Fig. 7. Diagrams showing changes of central hemodynamic variables evoked by i.v. infusion of adrenaline (0.1 and $0.3 \mu\text{g} \times \text{min}^{-1} \times \text{kg bwt}^{-1}$) in open-chest, vagotomized animals in the absence and presence of cardiac pacing ($250/\text{min}$). Observations were made on animals with intact adrenoceptors (open bars), after selective β_2 -adrenoceptor blockade (hatched bars), and after nonselective β -blockade (black bars). Observations were also made in the β_2 -blocked preparation after changes of cardiac afterload accomplished by a partial aortic occlusion (dotted bars). ☆, ★, £ indicate $p < 0.05$, see key.

(MAP) to those during adrenaline infusion after β_2 -blockade, led to clear-cut reductions of CO and SV despite a concomitant increase in LVEDP. After nonselective β -blockade (black bars), finally, the increases in max dP/dt evoked by adrenaline tended to be lower than after β_2 -blockade while the increases in LVEDP tended to be higher.

The conclusion may be drawn from these results that β_2 -blockade did not interfere with the adrenaline induced increase in the inotropic state. Therefore the reduced increase in CO after β_2 -blockade (which was not caused by a fall in preload since LVEDP actually increased) can be ascribed to the prominent increase in TPR and MAP, i.e. to increased afterload, in turn due to abolition of the β_2 -adrenergic peripheral vasodilator effect.

HR responses to adrenaline in closed-chest atropinized cats

The failing tachycardia response to adrenaline in the vagotomized open-chest cats after selective β_2 -blockade (Fig. 7) could, among other things (cf. Comments), be due to a baroreceptor mediated decrease in the sympathetic discharge to the heart which apparently was high in the control state in the open-chest preparation (cf. paper IV, Table 1). Such sympathetic reflex decrease in HR might be expected to be smaller in more 'atraumatic' preparations with lower resting sympathetic outflow. This possibility seemed supported by the results obtained in atropinized closed-chest cats (Fig. 8), in which HR, in response to adrenaline, increased not only in the preparation with intact adrenoceptors (from 195 ± 12 beats/min) but also after β_2 -blockade (from 188 ± 8 beats/min) in a dose dependent manner, despite the fact that there were pronounced increases in MAP which could have triggered baroreceptor responses. The dose-response curve, however, was somewhat shifted to the right after β_2 -blockade (see Comments).

Effects of noradrenaline in closed-chest cats with intact vagal nerves

The central hemodynamic response pattern to graded i.v. infusions of noradrenaline in closed-chest animals with intact vagal nerves in the absence and presence of selective β_2 - and nonselective β -adrenoceptor blockade were described in Fig. 4, paper IV. The results will be briefly summarized here. With intact adrenoceptors, noradrenaline evoked only minor changes of CO due to the combined effects of decreasing HR and increasing SV. TPR exhibited a dose-dependent and pronounced increase,

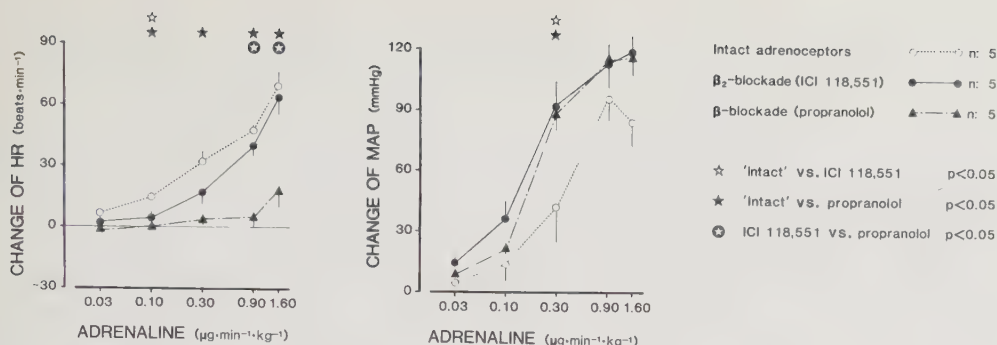


Fig. 8. Diagrams showing changes of HR and MAP evoked by i.v. infusions of adrenaline in graded doses on closed-chest, atropinized animals. Observations were made on animals with intact adrenoceptors (open circles), after selective β_2 -adrenoceptor blockade (closed circles), and after nonselective β -blockade (closed triangles). ☆, ★, ⊙ indicate $p < 0.05$, see key.

and MAP a corresponding rise. β_2 -blockade did not significantly alter this central hemodynamic response pattern to noradrenaline, implying that the responses to noradrenaline, in contrast to those evoked by adrenaline were little dependent on β_2 -adrenoceptor activation. In fact, the response pattern to noradrenaline in animals with intact adrenoceptors mimicked that evoked by adrenaline after selective β_2 -adrenergic blockade.

COMMENTS

An attempt was made in these expts to analyse the importance of cardiovascular β_2 -adrenoceptor stimulation for cardiac performance during graded i.v. administration of adrenaline, and also of noradrenaline. The net effect on cardiac performance of adrenoceptor activation by adrenaline was, in contrast to that of noradrenaline, to a large extent determined by β_2 -adrenoceptor stimulation. This was only little dependent on a direct β_2 -adrenergic effect on the heart (see below), but rather it was an indirect effect on cardiac afterload accomplished by β_2 -adrenoceptor dilator interaction with the concomitant α -adrenergic constriction of the systemic resistance vessels. The resulting net decrease in TPR to moderate (apparently 'physiological') doses of adrenaline (up to $0.3 \mu\text{g} \times \text{min}^{-1} \times \text{kg}^{-1}$, corresponding to a plasma concentration of about 20 nM) permitted CO to increase without an undue increase in MAP, i.e. in left ventricular afterload. It may be concluded that such maintenance of a low, roughly normal, afterload by the β_2 -adrenergic vasodilator effect is essential for eliciting 'optimal' increases in CO in response to adrenaline (Fig. 7). This conclusion is in agreement with that derived from previous observations during infusion of the syntheti

catecholamine, isoprenaline, showing that a superimposed experimental increase in the afterload (by administration of a vasoconstrictor agent) led to a less pronounced increase in CO (Liedtke, Buonchristiani, Kirk, Sonnenblick & Urschel 1972). It is quite likely that such an adrenaline induced β_2 -adrenergic influence on cardiac afterload is of even greater importance during hemorrhage when the inotropic state of the heart might be jeopardized by possible coronary flow restriction.

An indication of a direct effect on the heart of β_2 -adrenoceptor stimulation by adrenaline was obtained in terms of a moderate chronotropic response (Fig. 8) while left ventricular inotropy apparently was unaffected (Fig. 7). This is in consonance with results from pharmacological studies showing that β_1 - and β_2 -adrenoceptors co-exist in the heart (Carlsson et al. 1972, Åblad et al. 1974, Yabuuchi, Yamashita & Tei 1977), though to a functionally significant extent only in the sinus node region (Carlsson et al. 1977). Radio-ligand binding studies in several species have largely confirmed such a distribution of β_2 -receptors within the heart (Hedberg, Minneman & Molinoff 1980, Engel et al. 1981, Brodde, Leifert & Krehl 1982). In the cat about 25% of the β -adrenoceptors in the right atrium were reported to be of the β_2 -subtype, but only 2% in the left ventricle (Hedberg et al. 1980). It could be argued in this context that the shift to the right of the HR dose-response curve to adrenaline after administration of compound ICI 118,551 (Fig. 8) might be the result of β_1 - rather than β_2 -adrenoceptor blockade, implying, then, a relatively low β_2 -selectivity of the drug. This possibility, however, seemed to be refuted by the findings that this antagonist, in the dose used in the present study, did not interfere with the tachycardia induced by stimulation of the cardiac sympathetic nerves (Bilski et al. 1983) and, further, that HR can increase to a 'maximal' level during very strong hemorrhage induced reflex sympatho-adrenal activation in ICI 118,551 blocked cats (Fig. 10, section 5). Note also that the inotropic response (max dP/dt), mediated by adrenaline via β_1 -adrenoceptors, was equally large in the absence and presence of this drug (Fig. 7). It cannot be excluded, however, that a baroreceptor induced reflex decrease in sympathetic discharge, caused by a greater rise in MAP by adrenaline in the β_2 -blocked than in the preparation with intact adrenoceptors (Fig. 8), might have contributed, together with blockade of cardiac β_2 -adrenoceptors, to producing the shift in the HR dose-response curve (Fig. 8).

4. MICROSPHERE ANALYSIS OF β_2 -ADRENERGIC CONTROL OF RESISTANCE IN DIFFERENT VASCULAR AREAS IN HEMORRHAGE

Measurement in bleeding of TPR in cats with intact and with selectively blocked β_2 -adrenoceptors (section 2) revealed that hemorrhage is associated with a significant β_2 -adrenergic dilator interaction with constrictor influences on the systemic resistance vessels. An attempt was made in these expts (paper V) to define the vascular areas responsible for this β_2 -adrenergic effect on TPR in hemorrhage and, further, to investigate whether the β_2 -adrenergic resistance control may result in a redistribution of cardiac output. For this purpose the microsphere technique was used which offered the advantage of simultaneous, 'atraumatic' measurements of arterial blood flow to a large number of tissues. It also permitted determinations of CO, SV, and TPR (HR determined from the arterial pressure recording). Two differently labelled microspheres were used allowing two measurements to be made in each animal. Hemorrhage in all these expts was accomplished by rapid withdrawal of 15 ml blood $\times$ kg bwt^{-1} .

Effects on regional resistances of hemorrhage per se in animals with intact adrenoceptors

The pattern of vascular resistance response evoked by hemorrhage was studied in cats with intact β -adrenoceptors ($n=5$) by determination of regional vascular resistances in the prehemorrhage control period and 25 min after completed exsanguination. Most tissues tended to increase vascular resistance in response to bleeding (paper V, Table 1) and this constrictor effect was especially marked in the pancreas (+133% above control), urinary bladder (+97%), omental (+106%) and subcutaneous (+145%) fat, skin (+96%), and the pad of the paw (+106%). The adrenal gland, on the other hand, showed a clear-cut decrease of resistance (-46%). The raised resistance in individual tissues was reflected in TPR which increased by 28%. Concomitantly there was a decrease in both CO (-36%) and in MAP (-22%). These data for vascular resistances in the control period and after hemorrhage, although referring to a limited number of expts, are in good agreement with previous reports in the literature (Sapirstein, Sapirstein & Bredemeyer 1960, Neutze, Wyler & Rudolph 1968, Kaihara et al. 1969, Forsyth, Hoffbrand & Melmon 1970, Slater et al. 1973). As shown by the results below, the observed resistance responses to bleeding in several of the studied tissues

apparently reflected the net effect of concomitant vasoconstrictor and β_2 -adrenergic vasodilator influences on the resistance vessels.

β_2 -adrenergic effects on regional vascular resistances in hemorrhage

The possible β_2 -adrenergic dilator interaction with the hemorrhage induced vasoconstrictor response was examined in 9 cats by instituting 'acute' selective β_2 -blockade by i.v. administration of compound ICI 118,551 25 min after the exsanguination. Microsphere injections were made immediately before, and 10 min after application of the blocking agent. Acute β_2 -blockade caused a statistically significant increase in resistance in several tissues (paper V, Table 2), viz. in the stomach (+26%), small (+25%) and large (+38%) intestine, pancreas (+29%), kidney (+39%), omental (+33%) and subcutaneous (+26%) fat, gastrocnemius muscle (+19%), and skin (+24%). TPR also increased significantly (+19%) whereas CO decreased (-15%), mainly due to a decline in SV. These resistance data indicate the presence of a β_2 -adrenergic dilator component in the resistance response before blockade. Control expts (n=5) without β_2 -blockade showed no or only small 'spontaneous' changes in regional resistances during the 10 min period (from 25-35 min after exsanguination) which elapsed between the two abovementioned resistance determinations. The renal circuit was an exception, however, insofar that renal resistance rose significantly (+10%) in this time period; yet this effect was significantly smaller than that evoked by acute β_2 -blockade.

β_2 -adrenergic effects on fractional distribution of cardiac output in hemorrhage

As expected, β_2 -adrenergic blockade tended to decrease the fraction of CO distributed to tissues where the blocker caused a significant increase in regional resistance (paper V, Table 3). This effect, however, reached the level of significance only in the kidney, in which the fraction of CO decreased by 17% compared to the value observed with intact β_2 -adrenoceptors. In some other tissues β_2 -blockade instead caused a 'passive' increase in the received fraction of CO.

COMMENTS

The described findings indicate that bleeding is associated with a significant β_2 -adrenergic dilator interaction with the hemorrhage induced vasoconstrictor influences in several hemodynamically important tissues, viz. in the kidney, gastro-intestinal tract, pancreas, adipose tissue,

skin, and 'white' skeletal muscle. These effects also caused a net decline of TPR. The β_2 -adrenergic effect on the distribution of CO to the tissues was small, however, which might be explained by the fact that the dilator influence was present in a large number of tissues.

The presence of a β_2 -adrenergic inhibitory influence on vascular tone in the resistance vessels during bleeding has also been demonstrated by our previous studies of regional resistance in skeletal muscle, small intestine, skin, and kidney. In those expts, regional β -blockade was performed via i.a. administration of propranolol avoiding any systemic effect of the blocker. In such expts the observed increase in resistance by β -blockade must represent a strictly regional effect, in all probability reflecting a direct interaction with the vascular smooth muscle β -adrenoceptors. In the present study, on the other hand, the β_2 -blocking agent was administered systemically and it might be argued that it primarily could have produced a cardio-depressive effect with the observed decreased CO as a result and that the observed increase in resistance was a secondary, reflex phenomenon. It has been clearly shown, however, that the β_2 -blocking agent ICI 118,551, in the dose used, neither interferes with β_1 -adrenoceptors of the heart (cf. Methods and section 3) nor causes any nonspecific negative inotropic effect (for example due to a membrane-stabilizing activity; see Bilski et al. 1983). Mentioned should be made that a marked TPR increase in response to β_2 -blockade occasionally was observed without a concomitant decrease in CO. For example, in one of the present expts TPR increased by 21% concomitantly with an 8% increase in CO. In the series of expts described in section 2 (Fig. 5), 3 out of 7 animals showed a similar pattern of response to β_2 -blockade when instituted, as in this microsphere investigation, about 25 min after bleeding. TPR increased on the average by 15% in these expts (~30% blood loss) without a concomitant decrease in CO (+1% on the average). Such observations, taken together with the above described clear-cut evidence for a direct β_2 -adrenergic inhibition of vascular smooth muscle tone in individual vascular areas during hemorrhage, seem to warrant the conclusion that the raised resistance after β_2 -blockade is the primary effect and the decrease in CO a secondary phenomenon.

Isoprenaline has been reported to reduce arteriolar resistance in most vascular beds, but the magnitude of the dilator response varies in

different vascular areas and species differences seem to exist. The most pronounced isoprenaline induced inhibition of vascular tone is reported to be evoked in the gastro-intestinal tract and in phasic 'white' skeletal muscle (for ref. see Greenway 1981) but also skin can show prominent dilator responses, at least in the dog (Abboud, Eckstein & Zimmerman 1965). The present observations are in consonance with those findings.

The observed significant β_2 -adrenergic inhibition of vascular tone in the kidney and adipose tissue in the present expts deserves some additional comments. In the kidney isoprenaline is reported to produce only weak dilatation (for ref. see Aukland 1976, Greenway 1981) contrasting to the quite pronounced β_2 -adrenergic inhibition of vascular tone during hemorrhage, noted in the present expts. This discrepancy might tentatively be related to the possibility that the renal dilator response during isoprenaline infusion is counteracted by β_1 -adrenoceptor-mediated (cf. Keeton & Campbell 1980, Johns 1981) release of renin-angiotensin. The possible existence of such β_1 - as well as β_2 -adrenergic effects with opposing influences on the renal vasculature might also explain why we failed to reveal any major renal resistance effect during hemorrhage by applying regional nonselective β -blockade (Lundvall & Gustafsson 1981). In canine adipose tissue β -adrenergic inhibition of vascular tone is reported to be mediated by β_1 - rather than β_2 -adrenoceptors (Rosell & Belfrage 1979). The present clearcut β_2 -adrenergic resistance effect in the cat adipose tissue indicates that species differences might exist with regard to the vascular β -adrenoceptor subtype in this tissue. A recent report suggests that also in man significant dilator responses can be evoked in subcutaneous fat via activation of β_2 -adrenoceptors (Hjemdahl & Linde 1983). It cannot be excluded, however, that these effects in cat and man at least partly could represent an indirect, metabolic inhibition of vascular tone secondary to β_2 -adrenergic lipolysis. The presence of a β_2 -adrenergic lipolysis was indicated in the expts presented in section 5.

5. DECREASE IN SURVIVAL TIME IN β_2 -ADRENOCEPTOR BLOCKED CATS EXPOSED TO FATAL BLEEDING

The β_2 -adrenergic control of the peripheral circulation seems to contribute significantly to compensatory circulatory adjustments in hemorrhage. Thus, analysis of the central hemodynamics in hemorrhage indicated that β_2 -adrenergic PV restitution (section 1) leads to a gradual recovery of SV and, hence of CO (section 2). Further, β_2 -adrenergic vasodilator action contributed to a gradual restoration to control of an initially raised TPR. Such β_2 -adrenergic decline of TPR and MAP might help to promote CO (section 3) and overall tissue perfusion; capillary exchange might be further aided by the β_2 -adrenergic relaxation of precapillary 'sphincters' increasing the capillary surface area available for exchange (Hillman 1983). There was also some indication of a moderate β_2 -adrenergic redistribution of CO to some hemodynamically important tissues (section 4).

It appears that these seemingly beneficial β_2 -adrenergically mediated cardiovascular adjustments could contribute to improved survival in hemorrhage. This hypothesis was tested in this series of expts (paper VI) by performing a comparative study of the time of survival in cats with intact and selectively blocked β_2 -adrenoceptors exposed to fatal hemorrhage. A number of previous studies have indicated reduced survival in bleeding after nonselective β -blockade (Berk et al. 1967, Halmagyi, Gillett & Irving 1967, Zierott, Meyer & Lundsgaard-Hansen 1969 a, Zierott, Pappova & Lundsgaard-Hansen 1969 b), while a recent study could not reveal such an effect (Lefer, Lefer & Levin 1980). However, studies using nonselective blockade do not permit separation between vascular β_2 -adrenergic and cardiac β_1 -adrenergic influences, a problem overcome by the present approach using a selective β_2 -adrenergic blocking agent.

Survival time of cats with intact and selectively blocked β_2 -adrenoceptors exposed to fatal hemorrhage

The animals were exposed to standardized fatal bleeding instituted by an initial acute exsanguination of about 45% of the animal's total blood volume followed by an additional withdrawal of $1 \text{ ml} \times \text{kg bwt}^{-1} \times \text{h}^{-1}$ (for chemical analyses) throughout the time of survival. Comparative analyses were performed on 8 cats with intact and on 8 cats with selectively blocked β_2 -adrenoceptors. With blocked β_2 -adrenoceptors, the animals

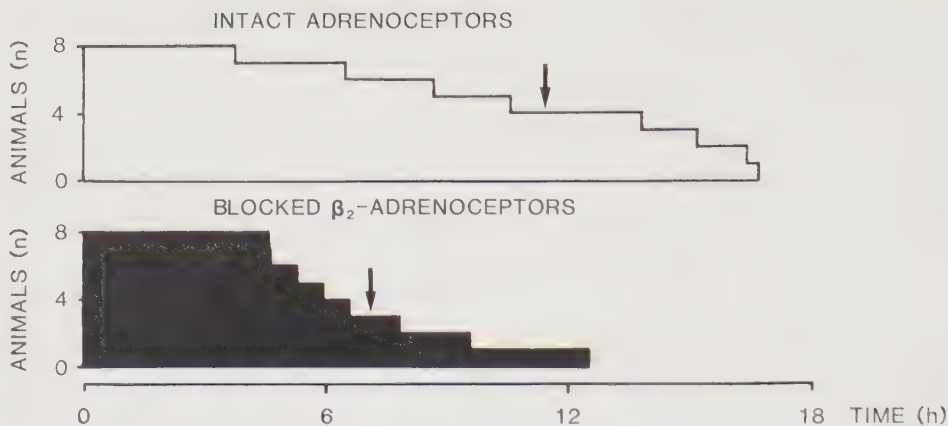


Fig. 9. Survival times after commencement of bleeding (23 ml $\times$ kg⁻¹) in animals with intact and selectively blocked β_2 -adrenoceptors. Ordinate shows number of animals. Arrows denote mean survival time in the two groups of animals.

exhibited a shorter survival time ($p < 0.05$) than animals with intact adrenoceptors. On the average, the β_2 -blocked cats lived for 427 min after commencement of hemorrhage and the animals with intact adrenoceptors for 686 min, the difference thus approaching 4.5 h, corresponding to a 38% reduction in survival time for the β_2 -blocked animals. Data for individual survival time are shown in Fig. 9.

Circulatory, respiratory, and metabolic variables in cats with intact and selectively blocked β_2 -adrenoceptor exposed to fatal hemorrhage

These data refer to changes, in the abovementioned two groups of animals, of MAP, HR, respiratory rate, arterial pO_2 , pCO_2 , pH, base excess, lactate, glucose, glycerol, osmolality, and hematocrit. Determinations of the circulatory, respiratory, and metabolic parameters were performed prior to bleeding and at intervals after hemorrhage. Determinations of adrenaline and noradrenaline plasma concentrations were made in the resting control state and 2 h after bleeding. The control data (paper VI, Table 1) for all these variables prior to bleeding were roughly similar (not significantly different) in the animals with intact and selectively blocked β_2 -adrenoceptors. A possible exception was the control plasma glycerol concentration which on the average was considerably lower in the latter group of animals.

Fig. 10 summarizes corresponding observations after bleeding (mean values of changes from the prehemorrhage control levels) for the animals with

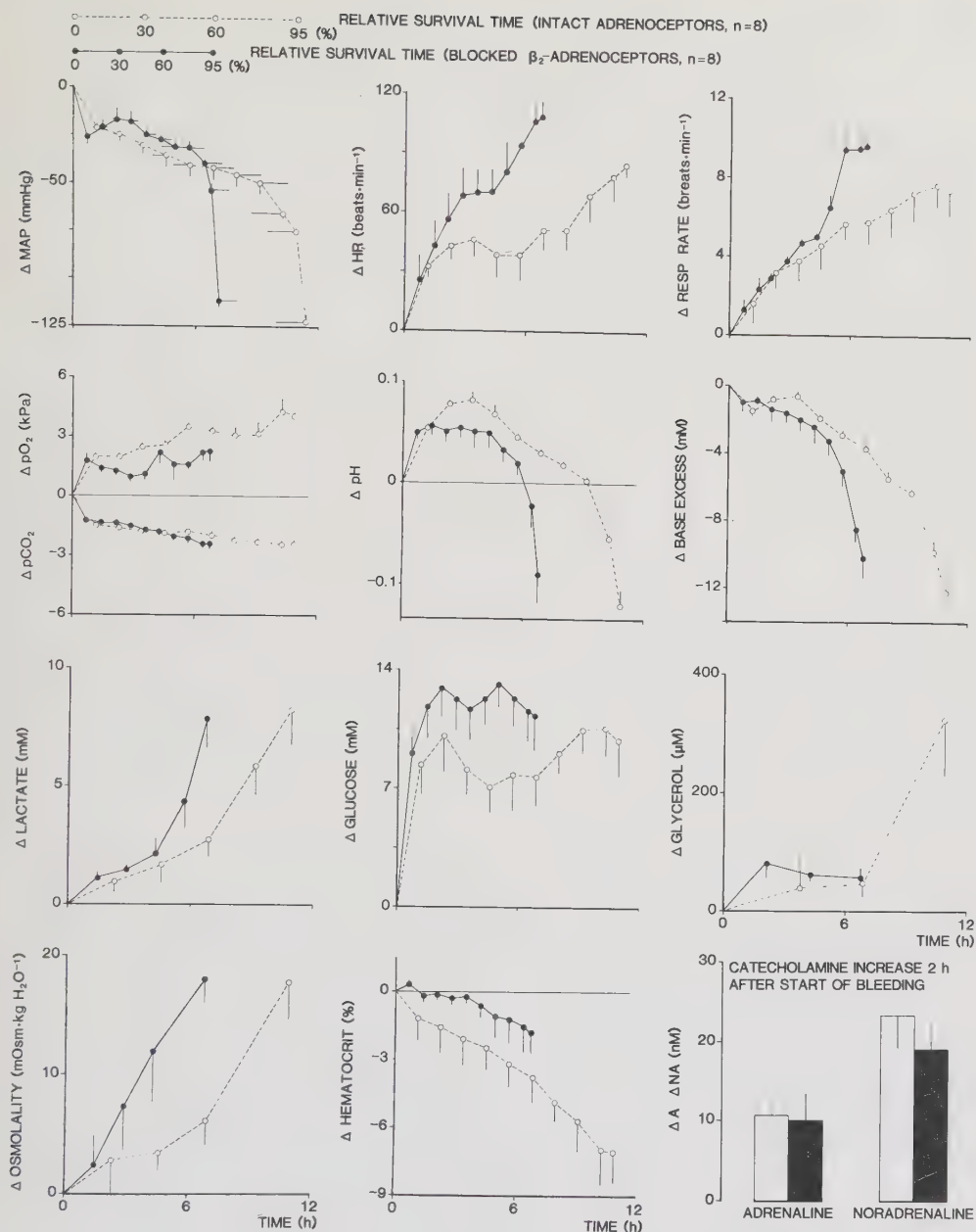


Fig. 10. Changes from prehemorrhage control levels of MAP, HR, and respiratory rate, and of arterial pO₂, pCO₂, pH, base excess, lactate, glucose, glycerol, osmolality, hematocrit, adrenaline and noradrenaline in exsanguinated animals with intact (open circles (bars)) and blocked β_2 -adrenoceptors (closed circles (bars)). Adrenaline and noradrenaline concentrations were only measured once (2 h) after commencement of bleeding.

intact and blocked β_2 -adrenoceptors. In an attempt to provide a representative illustration of average changes during the time of survival, the data were processed as described in detail in paper VI, Methods, implying that they were classed with regard to relative survival time in percent of total (see abscissa for relative survival time above the MAP diagram in Fig. 10). Thereby we circumvented the difficulty encountered by the fact that the number of observations decreased with time due to varying individual survival times. Such calculated mean data are plotted in the diagrams of Fig. 10 versus time (spread (SE) along the abscissa depicted only in the MAP diagram). Some comments will be made about the effects observed in the early stage, where relatively efficient compensation against hemorrhage seemed to occur in both groups of animals (up to about 4 h after commencement of bleeding), as well as in the later agonal stage.

The early stage (<4 h), was characterized by a moderate and similar decrease in MAP in both groups of animals and by clear-cut increases in heart rate and respiratory rate, the tachycardia response being somewhat more marked in cats with blocked than with intact adrenoceptors. Arterial blood pO_2 and pH increased, pCO_2 decreased, and base excess tended to decline to similar extents in both groups of animals. The plasma lactate, blood glucose and plasma glycerol concentrations, and the arterial plasma osmolality increased in both groups with a tendency to somewhat greater changes in the β_2 -blocked animals. Note that the glycerol concentrations in absolute terms were clearly lower in animals with blocked than with intact β_2 -adrenoceptors, due to the mentioned discrepancy in the prehemorrhage control state. Arterial hematocrit showed a clear-cut, gradual decrease in the animals with intact adrenoceptors but was largely unchanged in the β_2 -blocked ones. The changes of the plasma catecholamine concentrations determined 2 h after bleeding are depicted in the lower right panel of Fig. 10. The adrenaline concentration rose by about 10 nM and the noradrenaline concentration by about 20 nM in both groups of animals.

In the later, agonal stage of bleeding, more drastic changes in most of the measured variables occurred in both groups of cats but this stage, as mentioned, was reached much earlier for the animals with blocked β_2 -adrenoceptors than for those with intact ones. Otherwise, as can be seen in Fig. 10, these changes towards the end of survival were quite similar

in both groups with the exception that the glycerol concentration increased markedly in the animals with intact adrenoceptors but not in the β_2 -blocked cats. It might be noticed that arterial blood remained well oxygenated until death, which might be related to the increased respiration and to the ventilatory support applied to the animals in this study. Yet, there were signs of increased anaerobic metabolism and metabolic acidosis in the agonal stage signifying tissue hypoxia.

COMMENTS

The present comparative study of survival time after standardized fatal hemorrhage in anaesthetized cats with intact and selectively blocked β_2 -adrenoceptors showed that β_2 -blockade led to significantly shortened survival. The patterns of response in the agonal stage of both groups of animals were in several respects quite similar with signs of increased anaerobic metabolism, metabolic acidosis, and tissue hypoxia in spite of maintained arterial normoxia, signifying impaired nutritional blood supply to the tissues. The fact that this stage was reached much earlier in the β_2 -blocked animals might primarily be ascribed to the absence of the β_2 -adrenergic compensatory peripheral and central circulatory adjustments in hemorrhage mentioned in the introduction to this section.

It should be stressed, however, that β_2 -blockade might have interfered not only with such circulatory events but also with other, potentially essential (patho)physiological control mechanisms directly or indirectly linked to β_2 -adrenoceptor activity such as: capillary permeability (see section 1), pulmonary vascular β_2 -adrenoceptors (Hyman et al. 1981), blood cell aggregation (section 1), blood rheology (section 2), central (section 1) and peripheral (section 2) nervous autonomic activity, pulmonary respiratory function (for ref. see Persson et al. 1982), metabolism (see below), etc. Such possible interference is difficult to assess but seemed to be obvious with regard to the lipolytic activity in adipose tissue which was significantly decreased in the animals with blocked β_2 -adrenoceptors (paper VI, Table 1, and Fig. 10, above). This metabolic effect of the blocking agent might have contributed to the observed difference in survival time between the two groups of animals, especially in view of the fact that the substrate supply of free fatty acids and glycerol to the tissues is reported to be impaired by a hemorrhagic low flow state per se in adipose tissue (e.g. Rosell & Belfrage 1979).

DISCUSSION AND CONCLUSIONS

The aim of the present studies was to describe the central hemodynamic patterns of response to acute hemorrhagic hypovolemia in the absence and presence of systemic β_2 -adrenoceptor blockade, in an attempt to define the role of the β_2 -adrenergic control system in compensatory adjustments to bleeding. The results, taken together with our previous observations (see Introduction), may permit the following conclusions:

β_2 -adrenergic mechanisms appear to significantly promote the posthemorrhagic plasma volume restitution, mainly by facilitating the transcapillary fluid absorption process in skeletal muscle, and thereby improve circulatory filling and venous return. Such effects contribute to a gradual recovery of stroke volume and, hence, of cardiac output. β_2 -adrenergic dilator interaction with constrictor influences on the tone of the resistance vessels in several hemodynamically important circuits of the systemic circulation contributes to a gradual restoration to control in about 2 h of an initially raised peripheral resistance. The recovery of cardiac output and decrease in resistance appear to act in concert to improve tissue perfusion, further aided by reported β_2 -adrenergic microcirculatory adjustments enlarging the functional capillary surface area available for exchange. The maintenance of arterial hypotension, in part accomplished by the β_2 -adrenergic decline of resistance, is not necessarily disadvantageous, since the resulting decrease in cardiac afterload might facilitate the blood expulsion from the heart, an effect demonstrated to be evoked by adrenaline under normovolemia. A decreased afterload might be of even greater importance in hemorrhagic hypovolemia when cardiac contractility could be jeopardized by possible coronary flow restriction. Most of the β_2 -adrenergic influences on cardiac performance thus appear to be indirect, even if some direct β_2 -adrenergic chronotropic influence was revealed, as originally described by Carlsson et al. (1972). The described β_2 -adrenergic effects on central hemodynamics were graded in relation to the magnitude of the induced hemorrhagic hypovolemia, as expected for a true physiological regulatory system. The peripheral and central hemodynamic effects evoked by β_2 -adrenergic control seem to contribute importantly to the restoration of cardiovascular homeostasis after hemorrhage, as evidenced by the observed significantly longer survival time after fatal bleeding in animals with intact than with blocked β_2 -adrenoceptors.

Discussion relevant to the interpretation of the present results was presented in the Comments sections in the foregoing. A few aspects related to the present methodology and to previous literature deserve some further attention.

The present comparative analysis of cardiovascular responses in the absence and presence of systemically instituted selective β_2 -adrenoceptor blockade does not permit definite assessment of the sites of action of the β_2 -adrenergic control mechanism. Yet, from the considerations in the foregoing and in the original papers (I-VI) there is much to indicate that the described circulatory effects were mainly effected via β_2 -adrenergic actions directly on cardiovascular effectors, mainly vascular smooth muscle.

The present experimental model of bleeding by which a sudden fixed blood volume was withdrawn simulates a common type of acute hemorrhage in patients in which effective hemostasis is successfully instituted shortly after the blood loss. Such a well-defined exsanguination imposes on the experimental animal a given acute circulatory stress, which, via the various cardiovascular receptors, causes prompt reflex activation of the sympatho-adrenal system (cf. Chien 1967). This, in turn, evokes compensatory adjustments from which the animal can benefit with gradual more or less complete restoration of cardiovascular homeostasis as a result. This experimental bleeding technique therefore seems to offer quite optimal conditions for a detailed analysis of the subsequent compensatory central hemodynamic adjustments.

In many previous investigations another experimental bleeding model has been employed by which a fixed and maintained arterial hypotension level is achieved with the use of a pressure bottle connected to the arterial system of the animal (Werle, Cosby & Wiggers 1942, Lamson & DeTurk 1945). This experimental bleeding technique implies that the animal cannot benefit much from the physiological compensatory adjustments brought into play after bleeding. Hence, it causes an artificially prolonged and excessive circulatory stress to the animal which is more directly related to the arterial hypotension than to the hypovolemia per se. However, the loss of blood volume is a primary disturbance in hemorrhage and the decrease in arterial pressure only one of its secondary consequences. A third variety of experimental bleeding implies continuous withdrawal of

blood from the animal at a given rate. This technique provides no steady state and is reported to elicit much less of sympatho-adrenal activation than the two above mentioned methods (Hall & Hodge 1971). Such considerations may be taken to indicate that the acute, fixed volume, bleeding technique is the method of choice for the assessment of the compensatory cardiovascular adjustments studied in the present investigations.

Several previous studies have used nonselective β -blocking agents for investigations of the overall β -adrenergic influence on the cardiovascular system in hemorrhage (Berk et al. 1967, Goodyer 1967, Halmagyi et al. 1967, Zierott et al. 1969 a, b, Lefer et al. 1980, Hintze & Vatner 1982, Ushioda et al. 1983). Such studies, which permit no separation between cardiac β_1 - and vascular β_2 -adrenergic effects, have led to somewhat varying opinions of the role of the β -adrenergic control system in bleeding. At least partly, this might be ascribed to the different bleeding models employed, the varying severity of the bleeding, influence of anaesthesia, and species differences. In most studies, however, beneficial effects of the β -adrenergic control system were found, though most often interpreted as related to cardiac β -adrenoceptors. It should also be mentioned that β -adrenergic agonists have been shown to provide significant protection against death in hemorrhagic shock (Grega, Kinnard & Buckley 1967, Grega & Kinnard 1968).

The present study seems to be the first one aimed at determining specifically the role of β_2 -adrenergic control mechanisms in bleeding, demonstrating their great importance for cardiovascular restoration, at least in the cat. It might be noticed that the described beneficial peripheral and central circulatory effects evoked by the β_2 -adrenergic control system in fact are quite similar to those aimed at in modern shock therapy by transfusion and vasodilatator treatment.

SUMMARY

The present investigations on anaesthetized cats aimed at elucidating the functional significance of β_2 -adrenergic vascular control mechanisms for the central hemodynamic adjustments during acute hemorrhagic hypovolemia. Such central hemodynamic studies were prompted by our previous findings that sympatho-adrenal β_2 -adrenoceptor activation contributes importantly to the transcapillary fluid absorption process and other compensatory microcirculatory adjustments to hemorrhage (see review by Hillman 1983). The role of the β_2 -adrenergic component in the central hemodynamic responses was evaluated by performing comparative studies during bleeding on cats with intact and selectively blocked β_2 -adrenoceptors, using the highly selective β_2 -blocking agent ICI 118,551 as a pharmacological tool in the analysis.

The investigations referred to the β_2 -adrenergic effects on plasma volume restitution, cardiac output (CO), stroke volume (SV), heart rate (HR), mean arterial blood pressure (MAP), total peripheral resistance (TPR), and cardiac output distribution during graded hemorrhage. Adrenaline induced β_2 -adrenergic influences on cardiac chronotropy, inotropy, preload, and afterload were also studied in normovolemic cats, as well as the influence of β_2 -adrenoceptor blockade on the survival time of cats exposed to fatal bleeding. The following main conclusions were drawn:

Plasma volume (PV) restitution after hemorrhage was found to be significantly governed by β_2 -adrenergic control mechanisms. Thus about 50% of the shed PV, or about 35% of the shed blood volume, was restored 2 h after exsanguination of 20 and 40% of the total blood volume in animals with intact β_2 -adrenoceptors, whereas only about 14% of the shed PV, or about 8% of the shed blood volume, was restituted in animals with blocked β_2 -adrenoceptors. These data on PV expansion were compatible with the view that the fluid gain early after bleeding is mainly accomplished by transcapillary fluid absorption from the extravascular space of skeletal muscle, a process previously shown to be governed to a significant extent by β_2 -adrenergic microcirculatory adjustments.

The central hemodynamic responses to standardized hemorrhage were also influenced by β_2 -adrenergic mechanisms. In the initial stage after bleeding (up to 10 min) MAP, SV, and CO decreased and TPR increased to the

same extent in animals with intact and blocked β_2 -adrenoceptors. Later on, both groups exhibited the same arterial hypotension, but other hemodynamic variables were markedly different. In cats with intact adrenoceptors there was a gradual, partial recovery of SV and CO in the face of a restoration to control of TPR. In the β_2 -blocked cats, TPR continued to increase in the face of maintained low CO and declining SV. These patterns of response were roughly graded in relation to the extent of bleeding, tested for 10, 20, and 40% reductions of the animal's total blood volume. The recovery of SV and CO in animals with intact β_2 -adrenoceptors most likely can be attributed mainly to the demonstrated β_2 -adrenergic promoting effect on PV restitution, leading to improved circulatory and cardiac filling and, hence, to improved cardiac performance. The gradual decline of TPR in these animals could mainly be ascribed to a β_2 -adrenergic dilator interaction with the constrictor influences on the systemic resistance vessels, an effect which might improve tissue perfusion and, in addition, via decreased cardiac afterload might facilitate the expulsion of blood from the heart. β_2 -blockade eliminated such beneficial effects in bleeding.

β_2 -adrenergic influences on the heart and TPR were elucidated in greater detail by studying cardiac performance during infusion of the main endogenous β_2 -adrenoceptor agonist, adrenaline, in normovolemic cats. The main β_2 -adrenergic effect of adrenaline in physiological doses seemed to be to lower TPR, thereby preventing an undue increase in cardiac afterload which appeared essential for evoking 'optimal' increases in CO. Adrenaline, in addition, elicited a small direct β_2 -adrenoceptor mediated chronotropic response, but apparently no β_2 -adrenergic inotropic effect.

An attempt was made to elucidate with the microsphere technique whether the demonstrated β_2 -adrenergic effect on TPR in bleeding was caused by generalized β_2 -adrenoceptor activation within the systemic circulation or by an effect preferentially confined to a more limited number of tissues. In the latter case, the β_2 -adrenergic control might contribute to a redistribution of CO in bleeding. The results, referring to events about 30 min after the induced hypovolemia, proved no evidence of any major such redistributing effect, even if there was a tendency of a specially marked β_2 -adrenergic dilator effect in the kidney, possibly also in the pancreas, adipose tissue, and the gastrointestinal tract. There were no

signs of a β_2 -adrenergic vasodilator action within the central nervous system.

The described β_2 -adrenergic effects on central hemodynamics in bleeding, taken together with our previous findings of marked β_2 -adrenergic dilator effects in the microcirculation, suggest that the β_2 -adrenergic control system might improve nutritional tissue blood flow via increased PV, and hence venous return, and thereby increasing CO, the latter effect further facilitated via decreased TPR. In fact, these reflex β_2 -adrenergic circulatory events are similar to those aimed at in current shock therapy by transfusion and vasodilator treatment and might thereby favour survival. This hypothesis was tested by comparing the survival time in cats with intact and selectively blocked β_2 -adrenoceptors exposed to fatal hemorrhage. The results showed that the survival time after such bleeding was significantly (38%) shorter in cats with blocked than with intact β_2 -adrenoceptors. It therefore appears that β_2 -adrenergic compensatory peripheral and central circulatory adjustments in hemorrhage, possibly aided by other, e.g. metabolic, β_2 -adrenergic effects, are crucial for homeostasis and survival after severe bleeding.

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REFERENCES

- AARSETH, P. & BØ, G. 1972. Content of blood and of extravascular water in cat lungs during changes in total blood volume. *Acta Physiol Scand* 85:343-352.
- ABBOUD, F.M., ECKSTEIN, J.W. & ZIMMERMAN, B.G. 1965. Venous and arterial responses to stimulation of beta adrenergic receptors. *Am J Physiol* 209:383-389.
- ÅBLAD, B., CARLSSON, B., CARLSSON, E., DAHLÖF, C., EK, L. & HULTBERG, E. 1974. Cardiac effects of β -adrenergic receptor antagonists. The Myocardium. *Adv Cardiol* 12:290-302.
- ANDERSSON, P.-O., FARNEBO, L.-O., FREDHOLM, B.B., HAMBERGER, B., HOLST, J. & JÄRHULT, J. 1982. Metabolic and hormonal adjustments during hemorrhage in cats after interference with the sympatho-adrenal system. *Acta Physiol Scand* 114:111-119.
- ARMSTRONG Jr., G.G., PORTER Jr., H. & LANGSTON, J.B. 1961. Alteration of carotid occlusion response by anesthesia. *Am J Physiol* 201:897-900.
- AUKLAND, K. 1976. Renal blood flow. *Int Rev Physiol*. In: *Kidney and Urinary Tract Physiology II* (ed. K. Thurau), vol. 11, pp. 23-79. University Park Press, Baltimore.
- AXENBORG, J.E. & ELG, R.B. 1981. A computerized system for hemodynamic studies in experimental animals. In: *Proc 5th Nordic Meeting on Med Biol Eng* (eds P.Å. Öberg, E. Odeblad, J. Persson and O. Wigertz), vol. 1, pp. 117-119.
- BERK, J.L., HAGEN, J.F., BEYER, W.H., DOCHAT, G.R. & LA POINTE, R. 1967. The treatment of hemorrhagic shock by beta adrenergic receptor blockade. *Surg Gyn Obst* 125:311-318.
- BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. 1983. The pharmacology of a β_2 -selective adrenoceptor antagonist (ICI 118,551). *J Cardiovasc Pharmacol* 5:430-437.
- BORGDORFF, P. 1983. Peripheral resistance after cardiac output reduction in the barodenervated cat. *Circ Res* 52:7-15.
- BRAUNWALD, E. & ROSS Jr., J. 1970. Control of cardiac performance. In: *Handbook of Physiology* (eds R.M. Berne, N. Sperelakis and S.R. Geiger), sect. 2, vol. I, pp. 533-580. Williams & Wilkins, Baltimore.
- BRICK, I., HUTCHISON, K.J., McDEVITT, D.G., RODDIE, I.C. & SHANKS, R.G. 1968. Comparison of the effects of ICI 50172 and propranolol on the cardiovascular responses to adrenaline, isoprenaline and exercise. *Br J Pharmacol* 34:127-140.
- BRODDE, O.-E., LEIFERT, F.-J. & KREHL, H.-J. 1982. Coexistence of β_1 - and β_2 -adrenoceptors in the rabbit heart: Quantitative analysis of the regional distribution by (-)- ^3H -Dihydroalprenolol binding. *J Cardiovasc Pharmacol* 4:34-43.
- BUCKBERG, G.D., LUCK, J.C., PAYNE, D.B., HOFFMAN, J.I.E., ARCHIE, J.P. & FIXLER, D.E. 1971. Some sources of error in measuring regional blood flow with radioactive microspheres. *J Appl Physiol* 31:598-604.
- CARLSSON, E., ÅBLAD, B., BRÄNDSTRÖM, A. & CARLSSON, B. 1972. Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. *Life Sci* 11:953-958.
- CARLSSON, E., DAHLÖF, C.-G., HEDBERG, A., PERSSON, H. & TÅNGSTRAND, B. 1977. Differentiation of cardiac chronotropic and inotropic effects of β -adrenoceptor agonists. *Naunyn-Schmiedeberg's Arch Pharmacol* 300:101-105.
- CHALMERS, J.P., KORNER, P.I. & WHITE, S.W. 1966. The control of the circulation in skeletal muscle during arterial hypoxia in the rabbit. *J. Physiol.* 184:698-716.
- CHIEN, S. 1967. Role of the sympathetic nervous system in hemorrhage. *Physiol Rev* 47:214-288.

- CHIEN, S., DELLENBACK, R.J., USAMI, S., BURTON, D.A., GUSTAVSON, P.F. & MAGAZINOVIC, V. 1973. Blood volume, hemodynamic, and metabolic changes in hemorrhagic shock in normal and splenectomized dogs. *Am J Physiol* 225:866-879.
- DAHLÖF, C. 1981. Studies on β -adrenoceptor mediated facilitation of sympathetic neurotransmission. *Acta Physiol Scand*, suppl 500:1-147.
- DJOJOSUGITO, A.M., FOLKOW, B. & KOVÁCH, A.G.B. 1968. The mechanisms behind the rapid blood volume restoration after hemorrhage in birds. *Acta Physiol Scand* 74:114-122.
- DOBBINS, D.E., SOIKA, C.Y., PREMEN, A.J., GREGA, G.J. & DABNEY, J.M. 1982. Blockade of histamine and bradykinin-induced increases in lymph flow, protein concentration, and protein transport by terbutaline in vivo. *Microcirc* 2:127-150.
- DOLE, W.P., JACKSON, D.L., ROSENBLATT, J.I. & THOMPSON, W.L. 1982. Relative error and variability in blood flow measurements with radiolabeled microspheres. *Am J Physiol* 243:H371-H378.
- DUNLOP, D. & SHANKS, R.G. 1968. Selective blockade of adrenoceptive beta receptors in the heart. *Br J Pharmacol Chemother* 32:201-218.
- ELIASSEN, E., FOLKOW, B., HILTON, S.M., ÖBERG, B. & RIPPE, B. 1974. Pressure-volume characteristics of the interstitial fluid space in the skeletal muscle of the cat. *Acta Physiol Scand* 90:583-593.
- ENGEL, G., HOYER, D., BERTHOLD, R. & WAGNER, H. 1981. ($\pm$) [125 Iodo]-cyanopindolol, a new ligand for β -adrenoceptors: Identification and quantitation of subclasses of β -adrenoceptors in guinea pig. *Naunyn-Schmiedeberg's Arch Pharmacol* 317:277-285.
- ERIKSSON, B.-M. & PERSSON, B.-A. 1982. Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection. *J Chromatogr* 228:143-154.
- FARNEBO, L.-O., HALLMAN, H., HAMBERGER, B. & JONSSON, G. 1979. Catecholamines and hemorrhagic shock in awake and anesthetized rats. *Circulatory Shock* 6:109-118.
- FARNSWORTH, P.N., PAULINO-GONZALEZ, C.M. & GREGERSEN, M.-I. 1960. F_{cells} values in the normal and splenectomized cat: Relation of F_{cells} to body size. *Proc Soc Exp Biol Med* 104:729-733.
- FITZSIMONS, J.T. 1972. Thirst. *Physiol Rev* 52:468-561.
- FORSYTH, R.P., HOFFBRAND, B.I. & MELMON, K.L. 1970. Redistribution of cardiac output during hemorrhage in the unanesthetized monkey. *Circ Res* 27:311-320.
- GAUER, O.H. & HENRY, J.P. 1963. Circulatory basis of fluid volume control. *Physiol Rev* 43:423-481.
- GAUER, O.H. & HENRY, J.P. 1976. Neurohormonal control of plasma volume. *Int Rev Physiol*. In: *Cardiovasc Physiol II* (eds A.C. Guyton and A.W. Cowley), vol. 9, pp. 145-190. University Park Press, Baltimore.
- GOODYER, A.V.N. 1967. Left ventricular function and tissue hypoxia in irreversible hemorrhagic and endotoxin shock. *Am J Physiol* 212:444-450.
- GREEN, J.F. 1977. Mechanism of action of isoproterenol on venous return. *Am J Physiol* 232:H152-H156.
- GREENWAY, C.V. 1979. Effects of sodium nitroprusside, isosorbide dinitrate, isoproterenol, phenotamine and prazosin on hepatic venous responses to sympathetic nerve stimulation in the cat. *J Pharmacol Exp Ther* 209:56-61.
- GREENWAY, C.V. 1981. Mechanisms and quantitative assessment of drug effects on cardiac output with a new model of the circulation. *Pharmacol Rev* 33:213-251.
- GREGA, G.J. & KINNARD, W.J. 1968. Protective effects of nylidrin and isoproterenol against hemorrhagic shock. *J Pharmacol Exp Ther* 161:98-110.
- GREGA, G.J., KINNARD, W.J. & BUCKLEY, J.P. 1967. Effects of nylidrin,

- isoproterenol and phenoxybenzamine on dogs subjected to hemorrhagic shock. *Circ Res* 20:253-261.
- GREISHEIMER, E.M. 1965. The circulatory effects of anesthetics. In: *Handbook of Physiology* (eds W. Hamilton and P. Dow), sect. 2, vol. III, pp. 2477-2510. Am Physiol Soc, Washington D.C.
- GROOM, A.C. & ROWLANDS, S. 1958. The cardiac output and blood volume of the anaesthetized cat. *Phys Med Biol* 3:138-156.
- GROOM, A.C., ROWLANDS, S. & THOMAS, H.W. 1965. Some circulatory responses to haemorrhage in the cat: A critical level of blood volume for the onset of hypotension. *Quart J Exp Physiol* 50:385-405.
- HALES, J.R.S. 1974. Radioactive microsphere techniques for studies of the circulation. *Clin Exp Pharmacol Physiol*, suppl 1:31-46.
- HALL, R.C. & HODGE, R.L. 1971. Changes in catecholamine and angiotensin levels in the cat and dog during hemorrhage. *Am J Physiol* 221:1305-1309.
- HALL, J.E., SCHWINGHAMER, J.M. & LALONE, B. 1976. Mechanisms of blood vessel constriction during hemorrhage. *Am J Physiol* 230:569-578.
- HALMAGYI, D.F.J., GILLET, D.J. & IRVING, M.H. 1967. Partial and "complete" adrenergic blockade in posthemorrhagic shock. *J Appl Physiol* 22:487-494.
- HEDBERG, A., MINNEMAN, K.P. & MOLINOFF, P.B. 1980. Differential distribution of beta-1 and beta-2 adrenergic receptors in cat and guinea pig heart. *J Pharmacol Exp Ther* 212:503-508.
- HERWAARDEN van, C.L.A., FENNIS, J.F.M., BINKHORST, R.A. & LAAR van't, A. 1977. Haemodynamic effects of adrenaline during treatment of hypertensive patients with propranolol and metoprolol. *Europ J Clin Pharmacol* 12:397-402.
- HEYMANN, M.A., PAYNE, B.D., HOFFMAN, J.I.E. & RUDOLPH, A.M. 1977. Blood flow measurements with radionuclide-labeled particles. *Prog Cardiovasc Diseases* 20:55-79.
- HILLMAN, J. 1981. Further studies on beta-adrenergic control of transcapillary fluid absorption from skeletal muscle to blood during hemorrhage. *Acta Physiol Scand* 112:281-286.
- HILLMAN, J. 1983. Beta₂-adrenergic control of transcapillary fluid absorption and plasma volume in hemorrhage. *Acta Physiol Scand*, suppl 516:1-62.
- HILLMAN, J. & LUNDVALL, J. 1980. Beta-adrenergic dilator interaction with the constrictor response in resistance vessels of skeletal muscle during hemorrhage. *Acta Physiol Scand* 108:77-83.
- HILLMAN, J. & LUNDVALL, J. 1981 a. Hormonal and neurogenic adrenergic control of the fluid transfer from skeletal muscle to blood during hemorrhage. *Acta Physiol Scand* 112:271-280.
- HILLMAN, J. & LUNDVALL, J. 1981 b. Classification of β -adrenoceptors in the microcirculation of skeletal muscle. *Acta Physiol Scand* 113:67-71.
- HINTZE, T.H. & VATNER, S.F. 1982. Cardiac dynamics during hemorrhage. Relative unimportance of adrenergic inotropic responses. *Circ Res* 50:705-713.
- HJEMDAHL, P. & LINDE, B. 1983. Influence of circulating NE and Epi on adipose tissue vascular resistance and lipolysis in humans. *Am J Physiol* 245:H447-H452.
- HYMAN, A.L., NANDIWADA, P., KNIGHT, D.S. & KADOWITZ, P.J. 1981. Pulmonary vasodilator responses to catecholamines and sympathetic nerve stimulation in the cat. Evidence that vascular β 2-adrenoceptors are innervated. *Circ Res* 48:407-415.
- IMAI, Y., SATOH, K. & TAIRA, N. 1978. Role of the peripheral vasculature in changes in venous return caused by isoproterenol, norepinephrine, and methoxamine in anesthetized dogs. *Circ Res* 43:553-561.
- JAKSCHIK, B.A., MARSHALL, G.R., KOURIK, J.L. & NEEDLEMAN, P. 1974.

- Profile of circulating vasoactive substances in hemorrhagic shock and their pharmacologic manipulation. *J Clin Invest* 54:842-852.
- JÄRHULT, J. 1975. Osmolar control of the circulation in hemorrhagic hypotension. An experimental study in the cat. *Acta Physiol Scand*, suppl 423:1-84.
- JÄRHULT, J., HOLMBERG, J., LUNDVALL, J. & MELLANDER, S. 1976. Hyperglycemic and hyperosmolar responses to graded hemorrhage. *Acta Physiol Scand* 97:470-475.
- JÄRHULT, J., LUNDVALL, J., MELLANDER, S. & TIBBLIN, S. 1972. Osmolar control of plasma volume during hemorrhagic hypotension. *Acta Physiol Scand* 85:142-144.
- JOHNS, E.J. 1981. An investigation into the type of β -adrenoceptor mediating sympathetically activated renin release in the cat. *Br J Pharmacol* 73:749-754.
- JOHNSSON, G. 1975. Influence of metoprolol and propranolol on hemodynamic effects induced by adreneline and physical work. *Acta Pharmacol Toxicol* 36, suppl V:59-68.
- JOHNSSON, G. & ÖBERG, B. 1968. Comparative effects of isoprenaline and nitroglycerin on consecutive vascular sections in the skeletal muscle of the cat. *Angiologica* 5:161-171.
- KAIHARA, S., RUTHERFORD, R.B., SCHWENTKER, E.P. & WAGNER Jr., H.N. 1969. Distribution of cardiac output in experimental hemorrhagic shock in dogs. *J Appl Physiol* 27:218-222.
- KEETON, T.K. & CAMPBELL, W.B. 1980. The pharmacologic alteration of renin release. *Pharmacol Rev* 32:81-227.
- LAMSON, P.D. & De TURK, W.E. 1945. Studies on shock induced by hemorrhage. A method for the accurate control of blood pressure. *J Pharmacol Exp Ther* 83:250-252.
- LANGER, S.Z. 1980. Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* 32:337-362.
- LARAGH, J.H. & SEALEY, J.E. 1973. The renin-angiotensin-aldosterone hormonal system and regulation of sodium, potassium, and blood pressure homeostasis. In: *Handbook of Physiology* (eds J. Orloff and R.W. Berliner), sect. 8, pp. 831-908.
- LAURELL, S. & TIBBLING, G. 1966. An enzymatic fluorometric micrometod for the determination of glycerol. *Clin Chim Acta* 13:317-322.
- LEFER, A.M., LEFER, D.J. & LEVIN, R.J. 1980. Actions of non-cardiodepressant β -adrenergic blocking agents in hemorrhagic shock. *Adv Shock Res* 3:167-174.
- LIEDTKE, A.J., BUONCHRISTIANI, J.F., KIRK, E.S., SONNENBLICK, E.H. & URSCHEL, C.W. 1972. Regulation of cardiac output after administration of isoproterenol and ouabain: interactions of systolic impedance and contractility. *Cardiovasc Res* 6:325-332.
- LUNDGREN, O., LUNDWALL, J. & MELLANDER, S. 1964. Range of sympathetic discharge and reflex vascular adjustments in skeletal muscle during hemorrhagic hypotension. *Acta Physiol Scand* 62:380-390.
- LUNDVALL, J. & GUSTAFSSON, D. 1981. Beta-adrenergic control of peripheral vascular resistance during increased sympatho-adrenal activity. *Acta Physiol Scand* 112:101-103.
- LUNDVALL, J. & HILLMAN, J. 1978. Fluid transfer from skeletal muscle to blood during hemorrhage. Importance of beta adrenergic vascular mechanisms. *Acta Physiol Scand* 102:450-458.
- LUNDVALL, J., HILLMAN, J. & GUSTAFSSON, D. 1982. β -Adrenergic dilator effects in consecutive vascular sections of skeletal muscle. *Am J Physiol* 243:H819-H829.
- LUNDVALL, J. & JÄRHULT, J. 1976. Beta adrenergic dilator component of the sympathetic vascular response in skeletal muscle. Influence on the micro-circulation and on transcapillary exchange. *Acta Physiol Scand*

96:180-192.

- MARCINIAK, D.L., DOBBINS, D.E., MACIEJKO, J.J., SCOTT, J.B., HADDY, F.J. & GREGA, G.J. 1977. Effects of systemically infused histamine on transvascular fluid and protein transfer. *Am J Physiol* 233:H148-H153.
- MELLANDER, S. 1960. Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta Physiol Scand* 50, suppl 176:1-86.
- MELLANDER, S. & ÖBERG, B. 1967. Transcapillary fluid absorption and other vascular reactions in the human forearm during reduction of the circulating blood volume. *Acta Physiol Scand* 71:37-46.
- MIDDLEMISS, D.N., BUXTON, D.A. & GREENWOOD, D.T. 1981. Beta-adrenoceptor antagonists in psychiatry and neurology. *Pharmacol Ther* 12:419-437.
- MILLER Jr., R.G. 1981. Simultaneous statistical inference. Second ed., pp. 1-299. Springer-Verlag, New York.
- MINNEMAN, K.P., PITTMAN, R.N. & MOLINOFF, P.B. 1981. β -Adrenergic receptor subtypes: properties, distribution, and regulation. *Ann Rev Neurosci* 4:419-461.
- NEUTZE, J.M., WYLER, F. & RUDOLPH, A.M. 1968. Changes in distribution of cardiac output after hemorrhage in rabbits. *Am J Physiol* 215:857-864.
- ÖBERG, B. 1964. Effects of cardiovascular reflexes on net capillary fluid transfer. *Acta Physiol Scand* 62, suppl 229:1-98.
- O'DONNELL, S.R. & PERSSON, C.G.A. 1978. β -adrenoceptor mediated inhibition by terbutaline of histamine effects on vascular permeability. *Br J Pharmacol* 62:321-324.
- O'DONNELL, S.R. & WANSTALL, J.C. 1980. Evidence that ICI 118,551 is a potent, highly β_2 -selective adrenoceptor antagonist and can be used to characterize beta-adrenoceptor populations in tissues. *Life Sci* 27:671-677.
- ORDWAY, G.A. & LONGHURST, J.C. 1983. Cardiovascular reflexes arising from the gallbladder of the cat. Effects of capsaicin, bradykinin, and distension. *Circ Res* 52:26-35.
- PARRATT, J.R. 1974. The haemodynamic effects of prolonged oral administration of oxyfedrine, a partial agonist at β -adrenoceptors: Comparison with propranolol. *Br J Pharmacol* 51:5-13.
- PERSSON, C.G.A., ERJEFÄLT, I., GREGA, G.J. & SVENSJÖ, E. 1982. The role of β -receptor agonists in the inhibition of pulmonary edema. *Ann NY Acad Sci*. In: Mechanisms of lung microvascular injury (eds A.B. Malik and N.C. Staub), vol. 384, pp. 544-556.
- PERSSON, C.G.A. & SVENSJÖ, E. 1983. Airway hyperreactivity and microvascular permeability to large molecules. *Eur J Resp Diseases*, suppl 131, 64:183-214.
- PIRKLE Jr., J.C. & GANN, D.S. 1976. Restitution of blood volume after hemorrhage: role of the adrenal cortex. *Am J Physiol* 230:1683-1687.
- REDFORS, S. 1983. Blood flow distribution and fluid and electrolyte transport in the small intestine during arterial hypotension. An experimental study in the cat. Thesis, pp. 1-46. Gothenburg
- REDFORS, S. & SJÖVALL, H. 1984. The importance of nervous and humoral factors in the control of vascular resistance, blood flow distribution and net fluid absorption in the cat small intestine during hemorrhage. *Acta Physiol Scand*. In press.
- REED, R.K. & WIIG, H. 1981. Compliance of the interstitial space in rats. 1. Studies on hindlimb skeletal muscle. *Acta Physiol Scand* 113:297-305.
- ROSELL, S. & BELFRAGE, E. 1979. Blood circulation in adipose tissue. *Physiol Rev* 59:1078-1104.
- RUDOLPH, A.M. & HEYMANN, M.A. 1967. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circ Res* 21:163-184.
- RUTLEN, D.L., SUPPLE, E.N. & POWELL Jr., W.J. 1981. β -Adrenergic

- regulation of total systemic intravascular volume in the dog. *Circ Res* 48:112-120.
- SAPIRSTEIN, L.A., SAPIRSTEIN, E.H. & BREDEMEYER, A. 1960. Effect of hemorrhage on the cardiac output and its distribution in the rat. *Circ Res* 8:135-148.
- SCHWINGHAMER, J.M., GREGA, G.J. & HADDY, F.J. 1970. Skin and muscle circulatory responses during prolonged hypovolemia. *Am J Physiol* 219:318-326.
- SHANKS, R.G. 1966. The effect of propranolol on the cardiovascular responses to isoprenaline, adrenaline and noradrenaline in the anaesthetized dog. *Br J Pharmacol* 26:322-333.
- SLATER, G.I., VLADECK, B.C., BASSIN, R., KARK, A.E. & SHOEMAKER, W.C. 1973. Sequential changes in distribution of cardiac output in hemorrhagic shock. *Surg* 73:714-722.
- SMITH, H.J., HALLIDAY, S.E. & ROUSE, W. 1983. The effects of a beta-2 selective adrenergic receptor antagonist (ICI 118,551) on twitch tension in cat soleus muscle. *J Pharmacol Exp Ther* 224:228-230.
- SVENSJÖ, E., PERSSON, C.G.A. & RUTILI, G. 1977. Inhibition of bradykinin induced macromolecular leakage from post-capillary venules by a β_2 -adrenoceptor stimulant, terbutaline. *Acta Physiol Scand* 101:504-506.
- USHIODA, E., NUWAYHID, B., KLEINMAN, G., TABSH, K., BRINKMAN III, C.R. & ASSALI, N.S. 1983. The contribution of the β -adrenergic system to the cardiovascular response to hypovolemia. *Am J Obstet Gynecol* 147:423-429.
- VIVEROS, O.H., GARLICK, D.G. & RENKIN, E.M. 1968. Sympathetic beta adrenergic vasodilatation in skeletal muscle of the dog. *Am J Physiol* 215:1218-1225.
- WARREN, D.J. & LEDINGHAM, J.G.G. 1978. Renal vascular response to haemorrhage in the rabbit after pentobarbitone, chloralose-urethane and ether anaesthesia. *Clin Sci Mol Med* 54:489-494.
- WATTS, D.T. 1965. Adrenergic mechanisms in hypovolemic shock. In: *Shock and Hypotension* (eds L.C. Mills and J.H. Mayer), pp. 385-391. Grune & Stratton, New York.
- WEINER, N. 1980. Norepinephrine, epinephrine, and the sympathomimetic amines. Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: *The pharmacological basis of therapeutics*, 6th ed. (eds A. Goodman-Gilman, L. Goodman and A. Gilman), pp. 138-210. MacMillan, New York.
- WERLE, J.M., COSBY, R.S. & WIGGERS, C.J. 1942. Observations on hemorrhagic hypotension and hemorrhagic shock. *Am J Physiol* 136:401-420.
- WINTHER, K., KNUDSEN, J.B. & GORMSEN, J. 1983. Platelets and adrenergic receptors. *J Int Soc Thrombosis Haemostasis*. IX International Congress on Thrombosis and Haemostasis, p. 366:1153. F.K. Schattauer, Stuttgart-New York.
- YABUCHI, Y., YAMASHITA, S. & TEI, S.-S. 1977. Pharmacological studies of OPC-2009, a newly synthesized selective beta adrenoceptor stimulant, in the bronchomotor and cardiovascular system of the anesthetized dog. *J Pharmacol Exp Ther* 202:326-336.
- ZIEROTT, G., MEYER, C. & LUNDGAARD-HANSEN, P. 1969 a. Effects of beta-receptor blockade on the course of experimental hemorrhagic shock. *Z Kreisl.-Forsch* 58:892-903.
- ZIEROTT, G., PAPPOVA, E. & LUNDGAARD-HANSEN, P. 1969 b. Combined adrenergic blockade in experimental hemorrhagic hypotension. *Pflügers Arch* 310:1-15.



